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Invasive potential of common carp (*Cyprinus carpio*) and Nile tilapia (*Oreochromis niloticus*) in American freshwater systems

Luis Zambrano, Enrique Martínez-Meyer, Naercio Menezes, and A. Townsend Peterson

Abstract: Nonnative fish introductions disrupt ecosystem processes and can drive native species to local extinction. Two of the most widespread, introduced species are the common carp (*Cyprinus carpio*) from Eurasia and the Nile tilapia (*Oreochromis niloticus*) from Africa. In North and South America, these introductions stem from aquaculture facilities, as well as historical introductions for recreational angling. An emergent field of ecological niche modeling provides robust predictions of the geographic potential of alien species to better understand their capacity to become established at broad scales. We used this modeling approach to produce spatially explicit predictions of the invasive potential of common carp and Nile tilapia in the Americas. Model predictions were tested using occurrence data for established populations in their native area and in the Americas. Results indicated that predictive power of niche models was high. Distributional potential of common carp in the Americas covers most temperate regions and high mountain tropical aquatic systems, whereas that of Nile tilapia is focused in the tropics and coast areas. The consequences of the potential establishment of these exotic species can be profound on native aquatic faunas, particularly on highly diverse regions such as the Amazon Basin and central Mexico.

Résumé : Les introductions de poissons non indigènes perturbent les processus écosystémiques et peuvent causer l'extinction locale d'espèces indigènes. Deux des espèces les plus généralement introduites sont la carpe commune (*Cyprinus carpio*) d'Eurasie et le tilapia du Nil (*Oreochromis niloticus*) d'Afrique. En Amérique du Nord et du Sud, ces introductions proviennent des établissements d'aquaculture, ainsi que des empoissonnements passés pour la pêche sportive. Un domaine en émergence de la modélisation des niches écologiques permet de faire des prédictions robustes sur le potentiel géographique d'espèces exotiques et ainsi de comprendre leur capacité à s'établir sur de larges espaces géographiques. Cette approche de modélisation nous a permis de faire des prédictions explicites du point de vue spatial concernant le potentiel d'invasion de la carpe commune et du tilapia du Nil dans les Amériques. Nous avons testé les prédictions du modèle à l'aide de données de présence de populations établies dans leur aire d'origine et dans les Amériques. Les résultats montrent que le pouvoir prédictif des modèles de niches sont élevés. Le potentiel de répartition géographique de la carpe commune dans les Amériques englobe la plupart des régions tempérées et les écosystèmes aquatiques tropicaux de haute montagne; en revanche, le tilapia du Nil se concentre surtout dans les tropiques et les régions côtières. L'établissement potentiel des ces espèces exotiques peut avoir de graves conséquences sur les faunes aquatiques indigènes, particulièrement dans les régions de forte diversité, telles que le bassin de l'Amazone et le centre du Mexique.

[Traduit par la Rédaction]

Introduction

Introductions of non-indigenous fishes can reduce diversity and modify local community dynamics in freshwater systems (Minns and Cooley 2000). Introductions can be intentional, such as in extensive aquaculture or with introductions of top predators for recreational fishing, or incidental, such as escape from aquaculture facilities or via shipping ballast water. Consequences of introductions can be profound; species diversity of native fish assemblages can be greatly reduced (Rahel 2000; Jackson 2002), and food web function can be disrupted (Vander Zanden et al. 1999). Population viability can be lowered because of predator–prey interactions, as in the case of sea lamprey (*Petromyzon*

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marinus) introductions to the Laurentian Great Lakes of North America (Mills et al. 2003).

Most research on fish introductions has focused on site-specific processes (e.g., loss of species, food web disruptions). A clear focus has been on top-down predator effects stemming from species introductions, such as the role of Nile perch (*Lates niloticus*) in the destruction of the native cichlid fauna of Lake Victoria (Barel et al. 1985). A broader perspective is necessary to better assess areas vulnerable to fish introductions, for example to set up policies for preventing or mitigating negative effects of introductions.

Common carp (*Cyprinus carpio*) and Nile tilapia (*Oreochromis niloticus*) introductions in North and South America have been extensive, but may not yet represent the full distributional potential of both species. This point becomes relevant because these species have important effects in aquatic ecosystems (Tapia and Zambrano 2003). Common carp increase suspended solids in the water column (Zambrano et al. 2001), reduce water transparency (Pinto et al. 2005) and macrophyte coverage (Zambrano and Hinojosa 1999), and decrease habitat heterogeneity for native species (Perrow et al. 1999). Nile tilapia alter trophic web structure by competing with other fish and preying on juveniles of other fish (Morgan et al. 2004) and amphibians (L. Zambrano, unpublished data).

The emerging field of ecological niche modeling has proved useful in developing robust predictions of the distributional potential of alien species (Peterson 2003). This approach focuses on modeling the geographic manifestation of species' ecological niches (i.e., the conjunction of ecological conditions within which a species is able to maintain populations without immigration) (Grinnell 1917; Hutchinson 1957). Software applications that relate georeferenced occurrence points to geographic information systems' (GIS) layers, representing environmental conditions of the landscape, have been developed to characterize the ecological niches of species and produce predictive distribution maps (Stockwell and Noble 1992; Stockwell and Peters 1999). With recent evidence that niches are highly conserved over evolutionary time periods (Peterson et al. 1999; Martínez-Meyer et al. 2004) and several successful applications (Peterson 2003), it is now clear that this approach offers excellent predictivity regarding species' geographic potential on landscapes beyond their native ones (Peterson and Robins 2003; Iguchi et al. 2004; Peterson et al. 2004).

Herein, we present an analysis of the invasive potential of carp and tilapia in the New World, based on ecological niche models from their native ranges. Detailed evaluations of the capacity of particular fish species for establishing and extending populations in new environments would help decision-makers evaluate risks and benefits of particular introductions. Indeed, under some circumstances, potential benefits of aquaculture could be overshadowed by costs of control or eradication of alien species causing ecological damage.

Materials and methods

Ecological niche models were based on georeferenced occurrence points drawn from diverse sources, including museum specimen records, the World Wide Web, a fish production atlas (Carta Nacional Pesquera 2004) and literature (see Acknowledgements). In all, 54 points were available for common carp and 76 for Nile tilapia on their native ranges, and 747 and 43 points in the Americas, respectively. All occurrences were georeferenced with Internet-based electronic gazetteers (http://www.fallingrain.com/world/index.html) to the nearest 0.01° of latitude and longitude.

Ecological niches were modeled using the Genetic Algorithm for Rule-set Prediction (GARP) (Stockwell and Peters 1999). This algorithm relates ecological characteristics of known occurrence points to those of points randomly sampled from the rest of the study region, seeking — in an evolutionary-computing environment — to develop decision rules that best summarize factors associated with the species' presence. Previous tests of GARP's ability to predict geographic distributions accurately have been successful (Peterson 2001; Peterson and Vieglais 2001; Peterson 2003), including previous applications to predicting fish distributions (Wiley et al. 2003; Iguchi et al. 2004; McNyset 2005).

In GARP, occurrence points are divided twice evenly into training and test data sets — that is, an initial 50% of the data points are set aside for a completely independent test of model quality (extrinsic testing data); of the remaining points, half are used for developing models (training data) and half are used for tests of model quality internal to GARP processing (intrinsic testing data). GARP works in an iterative process of rule selection, evaluation, testing, and incorporation or rejection; a method is chosen from a set of possibilities (e.g., logistic regression, bioclimatic rules), applied to the training data, and a rule is developed or evolved. Predictive accuracy is then evaluated based on 1250 points resampled (with replacement) from the interface testing data and on 1250 points sampled randomly from the study region as a whole. Rules may evolve by a number of means that mimic DNA evolution: point mutations, deletions, crossing over, etc. Changes in predictive accuracy from one iteration to the next are used to evaluate whether particular rules should be incorporated into the model, and the algorithm runs either 1000 iterations or until convergence.

All modeling in this study was carried out on a desktop implementation of GARP available for public download (http://www.lifemapper.org/desktopgarp). This program offers excellent flexibility in choice of predictive environmental–ecological GIS data coverages. In this case, initially, we used 15 data layers summarizing elevation, slope, aspect, flow accumulation, flow direction, and topographic index at a native pixel size of 1 arc-second (~1 km^2, obtained from the US Geological Survey's Hydro-1K data set; http://edcdaac.usgs.gov/gtopo30/hydro). We also used aspects of climate, including 1961–1990 annual means representing diurnal temperature range; freeze days; mean annual precipitation; solar radiation; maximum, minimum, and mean annual temperatures; vapor pressure; and wet days (all at 0.5° native resolution — ~2500 km^2), obtained from the Intergovernmental Panel on Climate Change (http://www.ipcc.ch). All environmental coverages were resampled to an intermediate resolution of 0.1° (10 km^2) prior to analysis. This set of environmental variables has been seen to be appropriate and adequate for modeling distributions in several studies (Peterson and Vieglais 2001; Peterson 2003).

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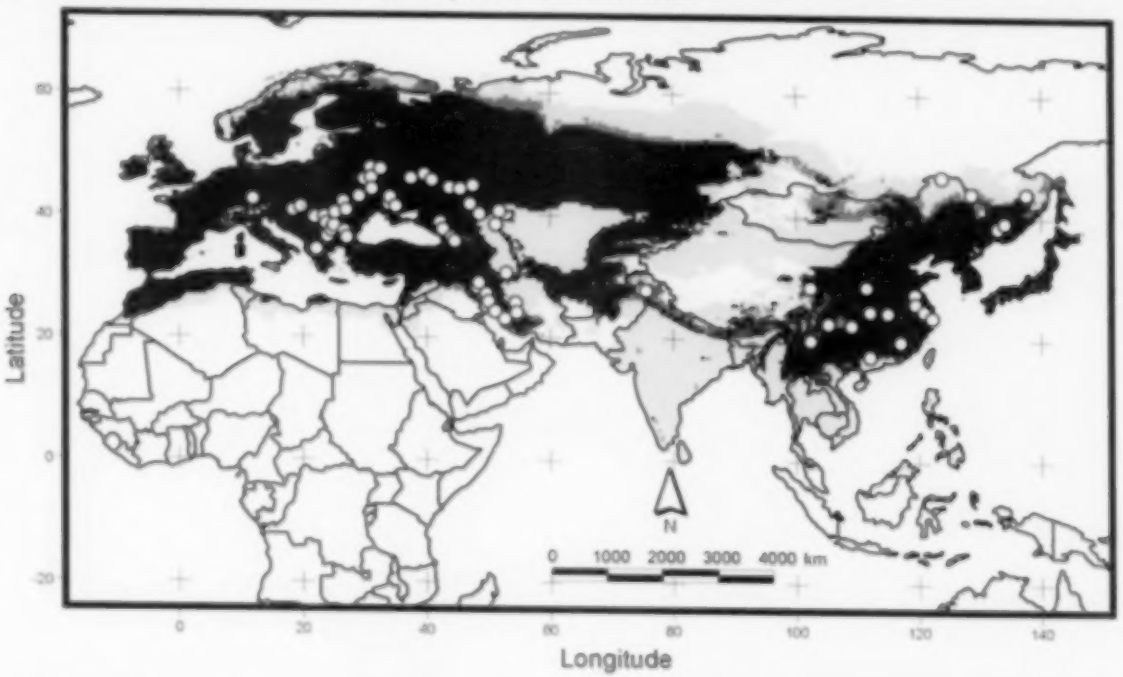
Fig. 1. Common carp (*Cyprinus carpio*) native distribution across Eastern Europe and Asia. Circles represent collection localities, and potential distribution is represented by shaded areas; light gray indicates low agreement (1–5), dark gray indicates medium agreement (6–8), and black indicates high agreement (9–10) among the 10 best-subset models.



To reduce environmental coverage sets to just those coverages that provide highest predictive accuracy, we used a series of jackknife manipulations (Peterson and Cohoon 1999). In general, we ran multiple iterations (1–20) of models omitting each coverage or each suite of coverages systematically. We then examined correlations between inclusion or exclusion of each coverage (binary coded) and omission error (percentage of extrinsic test presence data not predicted as present). Correlations on the order of $r > 0.1$ were considered indicative of strong detrimental contribution of a particular coverage to model quality, and such coverages were removed from further analyses. It is important to note that the jackknife manipulations were done solely on the native distribution of each species and so do not detract from the independent nature of the invaded-range predictions and tests presented herein.

To optimize model performance, we developed 100 replicate models of each species' ecological niche based on random 50–50 splits of available occurrence points and followed a modified procedure for extracting a "best subset" of the replicate models for further consideration (Anderson et al. 2003). The procedure is based on the observations that (*i*) models vary in quality, (*ii*) variation among models involves an inverse relationship between errors of omission (leaving out true distributional area) and commission (including areas not actually inhabited), and (*iii*) best models (as judged by experts blind to error statistics) are clustered in a region of minimum omission of independent test points and moderate area predicted (an axis related directly to commission error). The position of the cloud of points relative to the two error axes provides an assessment of the relative accuracy of the models. To choose best subsets of models, we (*i*) selected the 20 models with lowest omission error based on independent test points, (*ii*) calculated the median area predicted present among these low-omission models, and (*iii*) identified the 10 models that were closest to the median overall average area predicted present. A final map was created as the sum of these 10 best models (consensus map).

Model predictions were validated on native distributional areas (i.e., Africa for Nile tilapia, Asia for common carp) via the extrinsic testing data set. A χ^2 test was used to compare observed success in predicting distributions of test points with that expected under random models (the product of proportional area predicted present and number of extrinsic test presence points provides an estimate of occurrence points correctly predicted were the prediction to be random with respect to the distribution of the test points). Positive results in these tests would establish that models developed had sufficient predictive ability to be able to predict distributional phenomena for these species.

Projecting these models to the Americas provided predictions of potential geographic distributions of each species on its invaded range. We validated model predictions via overlaying known occurrence points for non-indigenous populations on the projections of the native-range models to the invaded distributional areas, using the same χ^2 approach as described above. To provide a binary prediction for these tests, we used 90% model agreement as a threshold for prediction of presence. To provide a more conservative test, we evaluated proportional area predicted present within a buffer of 750 km around the area predicted present.

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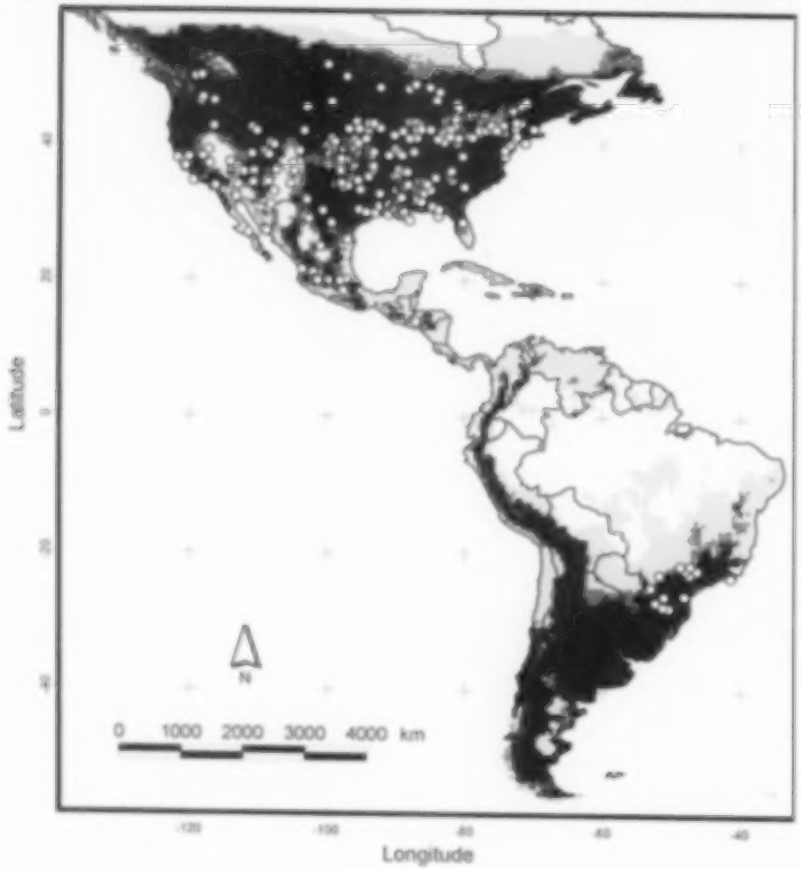
Fig. 2. Common carp (*Cyprinus carpio*) potential and known distribution in the Americas. Circles represent localities where this fish has been collected, and potential distribution is represented by shaded areas; light gray indicates low agreement (1–5), dark gray indicates medium agreement (6–8), and black indicates high agreement (9–10) among the 10 best-subset models.



Results

Common carp distribution

The native distribution of common carp covers a large area from Eastern Europe eastward across Russia and China (Fig. 1). In most of this area, temperature in winter is cold enough to freeze lakes, but in summer water reaches 25 °C, which is the temperature needed for carp reproduction (Matiland and Campbell 1992). Our models predicted distributional areas for carp across broad swaths of Asia (Fig. 1). Statistical tests indicate that models had much higher predictivity than random expectations (predictive success = 92.64%, $\chi^2 = 28.51$, df = 1, $P < 0.0001$).

Projection of native-range ecological niche models for common carp to the Americas shows broad areas with suitable conditions for the species. In North America, the predicted potential range includes most of Canada, the United States, and Mexico. Only the Sonoran Desert appeared inappropriate for carp (Fig. 2). In South America, areas prone to invasion are located principally in the south (Argentina, southern Brazil, and southern Chile; Fig. 2), but areas in the Andes suggest that opportunities exist even in tropical areas. Tests of model predictions for non-indigenous carp populations were significant both for North American populations (predictive success = 87.43%, $\chi^2 = 292.25$, df = 1, $P < 0.0001$) and South American populations (predictive success = 53.33%, $\chi^2 = 9.93$, df = 1, $P < 0.002$).

Nile tilapia distribution

The native distribution of Nile tilapia covers most of central Africa, limited to the north by the Sahara Desert and to the south in northern South Africa, where the limiting factor might be low air and water temperatures (Sifa et al. 2002). Our models predicted potential distributional areas across most of Africa except for the Sahara and the Kalahari deserts, but including the Mediterranean coast (Fig. 3). Model predictivity was high and statistically significant (predictive success = 87.48%, $\chi^2 = 17.5$, df = 1, $P < 0.001$).

Projection of the best-subset models to the Americas indicates suitable conditions for Nile tilapia establishment from the southeastern United States south along the coastal lowlands of Mexico and Central America (Fig. 4). In South America, however, the species' shows a broader geographic potential: from central Brazil to central Argentina, and from the eastern slopes of the Andes to the Atlantic Ocean, as well as parts of the coast of Venezuela and Guyana (Fig. 4).

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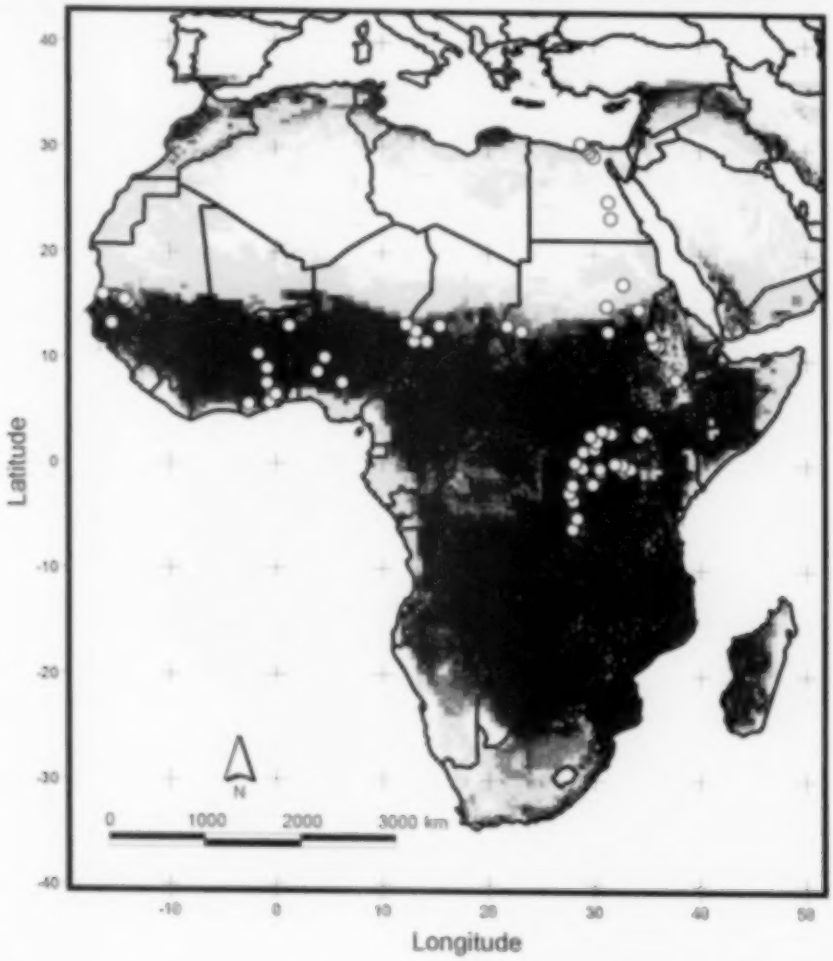
Fig. 3. Nile tilapia (*Oreochromis niloticus*) native distribution across the African continent. Circles represent localities where this fish has been collected, and potential distribution is represented by shaded areas; light gray indicates low agreement (1–5), dark gray indicates medium agreement (6–8), and black indicates high agreement (9–10) among the 10 best-subset models.



Tests of model predictions for non-indigenous Nile tilapia populations were significant both in North American populations (predictive success = 59.37%, $\chi^2 = 33.17$, df = 1, $P < 0.001$) and in South America populations (predictive success = 77.27%, $\chi^2 = 54.25$, df = 1, $P < 0.001$).

Discussion

The area that common carp or Nile tilapia may potentially inhabit in Asia, Africa, and the Americas indicates broad invasive potential. According to our results, common carp and Nile tilapia have a geographically extensive, invasive potential in the Americas, owing to their broad ecological niches. Common carp has the potential to establish in temperate systems, in both subtropical and temperate regions, and in the highlands within the tropics. Nile tilapia, on the other hand, has the potential to invade almost all tropical regions, particularly in the lowlands; indeed, this species is presently established in virtually every country in the Americas (Fitzsimmons 2001). Only the Sonoran Desert, northern Chile, and southeastern Argentina are likely to be unsuitable for both species.

The resulting invasive potential of these species in the Americas is a spatial representation of the appropriate environmental conditions (i.e., ecological niches) modeled in their native ranges. Hence, the veracity of the results depends on how well the GARP system was able to represent the niche of species, which in turn depends directly on the quality of the input data. For this study, we did not have available range-wide aquatic environmental data on factors that directly determine the presence of fish species (e.g., water temperature, pH, turbidity, etc.). Instead, we used atmospheric variables as proxies that have some limitations and may bias the results, as can be observed in the Nile tilapia, which was not predicted present in the Sahara. However, populations can be established there when water is available.

Ecological consequences of invasion and establishment of these species in local systems can be serious. Direct impacts

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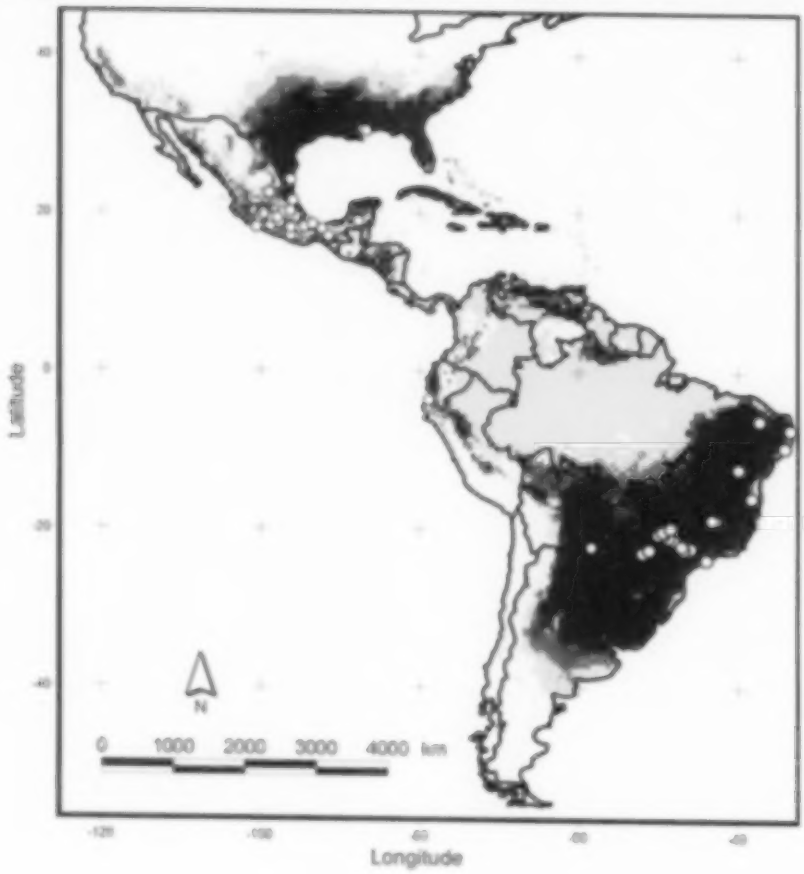
Fig. 4. Nile tilapia (*Oreochromis niloticus*) distribution in the Americas. Circles represent localities where this fish has been collected, and potential distribution is represented by shaded areas; light gray indicates low agreement (1–5), dark gray indicates medium agreement (6–8), and black indicates high agreement (9–10) among the 10 best-subset models.



of introduced common carp and Nile tilapia in natural systems include population depletion and even local extinctions of native species (Cahn 1929; Ogutu-Ohwayo 1990; Canonico et al. 2005). Furthermore, these species are known to affect the physical conditions of aquatic systems via increasing turbidity, changing concentrations of nutrients and suspended solids, and reducing spatial heterogeneity, particularly in rooted plants (Zambrano and Hinojosa 1999), altering the whole system structure and dynamics.

The Americas hold an impressive fish diversity, including >5400 freshwater species (Reis et al. 2003). Endemism reaches 30% in the United States, Mexico, and Chile. Together with Brazil, with its immense Amazonian diversity, these countries have seen the highest increases in Nile tilapia production in recent years (Fitzsimmons 2001). Nile tilapia can establish in systems dominated by American fish families such as Poecilidae (216 species), Cichlidae (406 species), and Characidae (952 species), which together include >1574 species (Reis et al. 2003). Similarly, carp invasion may jeopardize even entire endemic American families (e.g., Goodeidae, >40 species).

General considerations for aquaculture

In recent years, common carp and Nile tilapia aquaculture has increased in many American countries as a consequence of a crisis in the fisheries resulting from overfishing and reduction in shrimp culture (Aiken et al. 2002; Alceste et al. 2001). The economic strategy has tended towards a switch from species of high monetary value but high production cost (e.g., shrimp, *Litopenaeus vannamei*) to species of lower economic value but very low production cost (e.g., common carp, *C. carpio* and Nile tilapia *O. niloticus*). Under this scheme, common carp and Nile tilapia production has to be high to generate acceptable profit, increasing risk of negative impacts on the system. In some regions, this production system has been economically and socially successful; however, in many others, ecological consequences were counterproductive (Tapia and Zambrano 2003).

Concern about potential negative effects of introductions of alien fish species is minimal in most countries. Awareness of dangers of introductions and the potential for dire ecological and biodiversity consequences has developed only relatively recently, after decades of promotion of aquaculture as

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a solution to fulfill protein needs for human populations. Once these species are established in a system, eradication is extremely expensive and in many cases impossible. Hence, before releasing alien species in a system, it is necessary to estimate the potential success and counterbalance the ecological aftermath. This analysis should be mandatory in regions such as the Amazon Basin and central Mexico, where the potential for major loss of fish diversity is higher than any potential economical benefits.

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First-year growth and recruitment of coastal largemouth bass (*Micropterus salmoides*): spatial patterns unresolved by critical periods along a salinity gradient

Adam C. Peer, Dennis R. DeVries, and Russell A. Wright

Abstract: Although critical periods often explain first-year growth and recruitment patterns of young fishes, isolated subpopulations in spatially heterogeneous environments can be influenced by distinct factors, preventing critical periods from explaining growth and recruitment for the overall population. When we explored first-year growth and recruitment variability of largemouth bass (*Micropterus salmoides*) along an upstream–downstream gradient in the Mobile–Tensaw Delta, Alabama, USA, growth was consistently faster at sites closest to or within brackish habitats in 2002 and 2003, despite different abiotic conditions between years. Energetic content of food consumed by faster-growing fish, particularly those furthest downstream, was greater than that for slower-growing fish. Although the timing of the switch to piscivory did not explain growth differences, the degree of piscivory was important. Hatch date had no influence on growth differences and consequently did not affect fall condition, overwinter survival, or age-1 recruitment. Contrary to several studies in freshwater systems at this latitude, first-winter mortality was neither size-selective nor excessive, and largemouth bass continued to grow through the winter. These results demonstrate that early growth can vary substantially among subpopulations in spatially heterogeneous environments and that these differences are not necessarily explained by the same factors thought to be important for freshwater largemouth bass populations.

Résumé : Bien que les périodes critiques expliquent souvent les patrons de croissance et de recrutement de la première année chez les jeunes poissons, des sous-populations isolées dans des habitats hétérogènes quant à l'espace peuvent être influencées par des facteurs particuliers, ce qui peut empêcher les périodes critiques d'expliquer la croissance et le recrutement pour la population dans son ensemble. Lorsque nous examinons la variabilité en 2002 et 2003 de la croissance et du recrutement de première année chez l'achigan à grande bouche (*Micropterus salmoides*) le long d'un gradient de l'amont vers l'aval dans le delta de Mobile–Tensaw, Alabama, É.-U., la croissance est toujours plus rapide dans les sites qui sont situés le plus près des habitats d'eau saumâtre ou qui sont en eau saumâtre, malgré les conditions abiotiques différentes qui prévalent au cours de ces années. Le contenu énergétique de la nourriture consommée par les poissons qui croissent plus vite, particulièrement ceux le plus en aval, est plus élevé que celui des poissons à croissance plus lente. Bien que le moment du passage à l'ichtyophagie n'explique pas les différences dans la croissance, le degré d'ichtyophagie est important. La date de l'éclosion est sans effet sur les différences de croissance et, par conséquent, elle n'affecte pas la condition à l'automne, la survie à l'hiver, ni le recrutement de l'âge 1. Contrairement à ce que démontrent plusieurs études en eau douce aux mêmes latitudes, la mortalité durant le premier hiver n'est ni excessive, ni reliée à la taille; les achigans à grande bouche continuent d'ailleurs à croître durant l'hiver. Ces résultats montrent que la première étape de la croissance peut varier considérablement d'une sous-population à une autre dans les habitats hétérogènes quant à l'espace et que ces différences ne s'expliquent pas nécessairement par les mêmes facteurs que l'on croit importants pour les populations d'eau douce d'achigans à grande bouche.

[Traduit par la Rédaction]

Introduction

During the early life of many aquatic organisms, critical periods or events have important ramifications on growth, survival, and recruitment (Hjort 1914; Wehrtmann 1991; Underwood and Keough 2001). For many fishes such as the largemouth bass (*Micropterus salmoides*), recruitment success often is determined by one or more critical early-life

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periods, all of which cause or are related to differential growth or size of young fish. Critical periods for largemouth bass include time of hatching, onset of piscivory, fall lipid accumulation, and first winter survival (Ludsin and DeVries 1997). Evidence for these critical periods, however, has been found in relatively simple lentic systems, where the variability and rate of organism, material, and energy transport is considerably less complex than those in tidal freshwater and low salinity estuaries in which this species is also often found.

As ecotones between fresh water and ocean, estuaries possess a spatially and temporally more complex fauna than either downstream marine or upstream freshwater habitats (Peterson and Ross 1991). These ecosystems also exhibit extreme spatial and temporal variation in temperature, salinity, dissolved oxygen, discharge, turbidity, and nutrient concentrations, all of which can directly influence organism growth (Paperno et al. 2000; Manderson et al. 2002), survival (Paperno et al. 2000), and abundance. This spatial and temporal variation creates a mosaic of habitats that may produce substantial variation in vital rates. This variation in turn can create distinct subpopulations that are weakly linked (Lewis and Eby 2002) and thus differentially affected by specific ecological processes.

Although largemouth bass usually is recognized as a freshwater sport-fish species, coastal populations exist in low-salinity environments along the US coasts of the Atlantic Ocean and Gulf of Mexico. Coastal largemouth bass generally inhabit waters that range from fresh water to oligohaline (0.5–5.0 ppt) (Meador and Kelso 1989), although some individuals have been reported from salinities up to 24 ppt (Bailey et al. 1954). Often these coastal populations are abundant and can support popular recreational fisheries (Nack et al. 1993).

Despite largemouth bass presence and contribution to sport-fish harvests in coastal environments, there has been little work to assess the dynamics of these populations or to quantify juvenile growth and recruitment patterns across the highly variable habitats characteristic of coastal estuaries. Those studies in coastal systems that have included age-0 largemouth bass generally provided only limited insight on habitat use and distributions (e.g., Swingle and Bland 1974; Tucker 1985; Nack et al. 1993), and only one field study quantified individual growth of age-0 largemouth bass in low-salinity environments (Colle et al. 1976).

In this study, we examine patterns of growth and recruitment of age-0 largemouth bass in a tidal-freshwater (constant 0.1 ppt) to mesohaline (5–18 ppt) estuary along the northern Gulf of Mexico and test the ability of critical periods to explain observed patterns. Specifically, we quantify growth variability and identify the roles of both timing and degree of piscivory in explaining growth patterns. In addition, we evaluate whether variation in hatch date contributes to spatial growth variation in this system and whether growth and (or) hatch date influence overwinter survival and recruitment. In the process of describing the role of hatch date, piscivory, and overwinter survival, we build upon a conceptual model (i.e., Ludsin and DeVries 1997) to describe the interdependency of early life stages. Finally, we examine the potential nursery benefits of a low-salinity estuary to a freshwater predator and its management implications

Materials and methods

This study was conducted in the Mobile–Tensaw Delta (hereafter referred to as the Delta), Alabama, along the northern Gulf of Mexico. We established six sampling sites along a 20 km stretch of the Delta (Fig. 1) in an effort to maximize upstream–downstream (here these terms are not used literally, but instead refer to the upper and lower portions of the study area, respectively) geographic coverage and to incorporate predominant habitat types and physicochemical characteristics of this region (e.g., salinity; Fig. 2). Within our study area, the habitat shifted from seasonally flooded bottomland hardwoods dominated by bald cypress (*Taxodium distichum*) and tulepo gum (*Nyssa aquatica*) upstream to marsh habitat dominated by dense, emergent macrophytes (e.g., *Zizania* spp., *Phragmites* spp.) downstream.

Fish sampling

All fishes collected during this study were sampled monthly using boat-mounted and prod-pole (see below), DC-pulsed electrofishers (7.5 GPP, 7500 watt; Smith-Root, Vancouver, Washington) from June 2002 through April 2004. In 2002, biweekly sampling took place from May through August. Boat-mounted electrofishing was used to conduct two 15–20 min nearshore transects targeting larger fish. Because seining was impractical in these habitats, a hand-held prod-pole electrofisher was also used from the boat to conduct two to four 10–15 min inshore transects to target smaller age-0 largemouth bass and potential prey fishes. The handheld unit was composed of a 3.5 m telescoping pole with a 27 cm circular anode fitted with 0.4 cm mesh netting.

Each age-0 largemouth bass (hereafter called age-0 bass) collected was placed on ice and returned to the laboratory, where they were weighed (nearest 0.1 g) and measured (nearest mm, total length, TL). Otoliths and stomachs were removed, and the remaining fish tissue was frozen in water for later caloric density analyses. Otoliths were stored dry prior to mounting, and stomachs were placed in water and frozen for later analyses. All other fishes <80 mm were placed on ice and returned to the laboratory, where they were frozen for later identification and measurement of length and weight. All fish >80 mm and largemouth bass of sizes decidedly larger than an age-1 bass were weighed (nearest 0.1 g) and measured (nearest mm, TL) in the field and returned to the water.

Physicochemical sampling

Concurrent with fish sampling, salinity (YSI Model 30/50 FT meter; Yellow Springs Instruments Co., Yellow Springs, Ohio), water temperature (°C), and dissolved oxygen (YSI Model 51 B meter; Yellow Springs Instruments Co.) profiles were measured at 1 m depth intervals and the Secchi depth (cm) recorded. We attempted to measure water temperature at all sites every 2 h using temperature loggers at 1 m depth; however, during winter 2002–2003 and winter 2003–2004, temperature data were only available at Crab Creek (CC) and Dennis Lake (DL) because of the loss of loggers at other

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Fig. 1. Study area and sampling sites in the Mobile–Tensaw Delta, Alabama, USA: DB, D'Olive Bay; BM, Bay Minette; CC, Crab Creek; GI, Gravine Island; ML, McReynolds Lake; and DL, Dennis Lake.

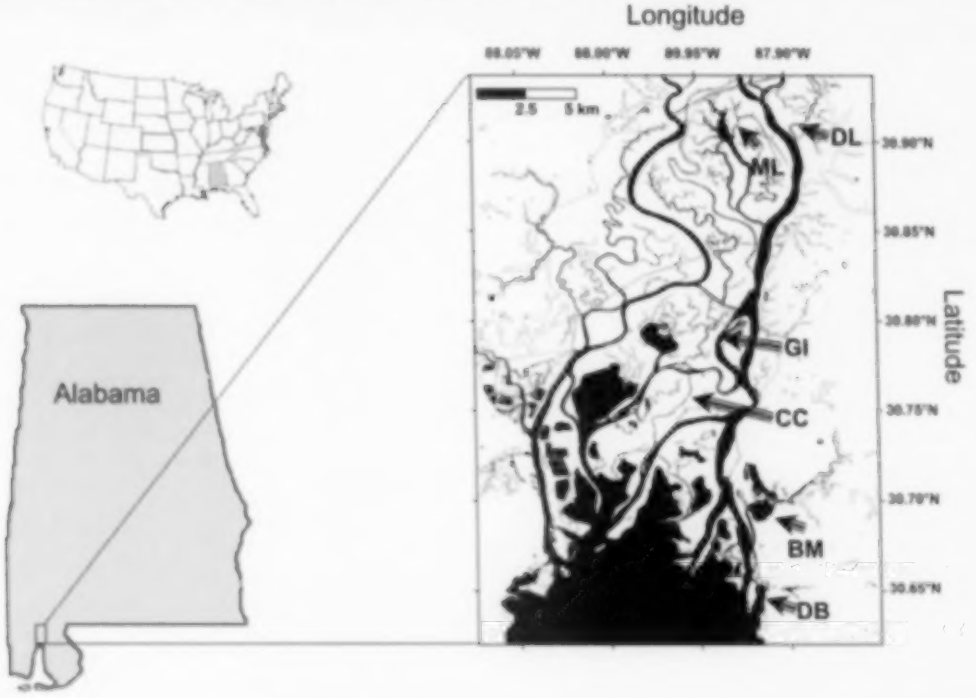
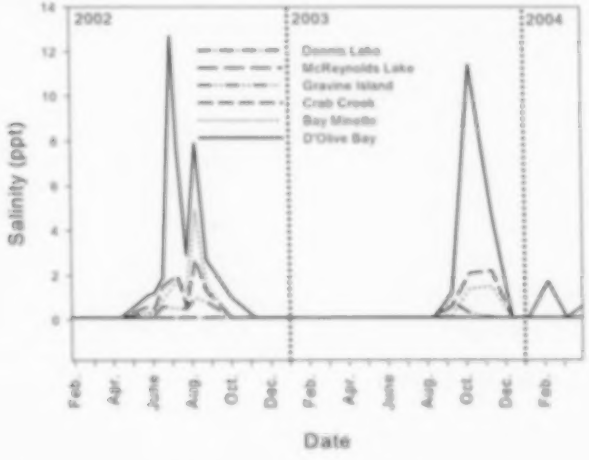


Fig. 2. Salinity (ppt) data collected at six sites in the Mobile–Tensaw Delta from January 2002 through March 2004. Salinity at Dennis Lake and McReynolds Lake were consistently 0.1 ppt throughout this study.



sites. Water samples were taken at each site to analyze turbidity (nephelometric turbidity units) in the laboratory.

Quantifying prey availability

To quantify fish prey availability during early summer (June–July) and late summer (August), prod-pole collections were used to estimate the relative abundance (catch·10 min transect^{-1}) of fish that age-0 bass were capable of consuming. Available fish prey were defined to be those *Leponis* spp. that were less than one-third of the mean bass TL (Lawrence 1958; Shelton et al. 1979) and all other species that were less than one-half of the mean bass TL as determined from age-0 bass TL – prey TL relationships observed in this study.

Diet composition, energetic content of food consumed, and transition to piscivory

Stomach contents of age-0 bass were examined from June to August of each year to quantify diet composition. A subsample of age-0 bass was taken by dividing the total catch at each site on each date into 5 mm length classes. When sample size permitted, a subsample of at least 20 fish per site per date was selected, such that the length–frequency distribution of the subsample matched the length–frequency distribution of the electrofishing samples within sites. Stomachs from fish were frozen for subsequent diet analyses. Stomach contents were identified to at least genus for fish, family for macroinvertebrates, and order for zooplankton. Biomass (mg·g dry mass^{-1}) of prey ingested by bass was quantified using prey length–mass regressions generated from fish and shrimp (i.e., daggerblade grass shrimp (*Palaemonetes pugio*) and white shrimp (*Litopenaeus setiferus*)) collected in this study and from literature estimates for zooplankton and benthic invertebrates (Pace and Orcutt 1981; Culver et al. 1985; Benke et al. 1999).

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To determine the relative energetic value of prey consumed by age-0 bass during early summer (June–July) and late summer (August), the calories (cal food-g bass^{-1}) in each stomach sample were quantified. Prey biomass estimates were converted to calories using caloric density values obtained from fish and shrimp collected during this study (see methods below) and from the literature for zooplankton and benthic invertebrates (Cummins and Wuycheck 1971; Driver et al. 1974; Lei and Armitage 1980).

Prior to determining caloric density values for potential prey items, samples were oven-dried (60 °C), pelleted, and ignited in a bomb calorimeter (Parr Instruments model 1425, semimicro bomb calorimeter; Parr Instruments, Moline, Illinois) to measure caloric content. Calibration with benzoic acid standards took place at 200-run intervals. When sufficient tissue was available, replicate samples from an individual were analyzed until two caloric densities were within 2% of one another. The caloric density for each individual was determined as the average of all caloric measurements for that sample.

Age-1 recruitment

Catch per unit effort (CPUE) of age-1 largemouth bass collected during the combined months of March and April was used as an indicator of recruitment. CPUE estimates were calculated at each site in spring 2003 and 2004 using prod-pole and boat-mounted electrofishing. Both sampling techniques efficiently caught age-1 largemouth bass at all sites. Combining both fishing gears offered greater coverage and more accurate relative abundance estimates, given that each sampled a different portion of the shoreline.

Hatch date and growth during spring and early summer

To determine age and quantify swim-up dates and daily growth rates, otoliths were removed from a subsample of age-0 bass from each site on two dates each summer (17–19 June 2002, 9–12 July 2002, 16–18 June 2003, and 14–16 July 2003). As described above, the length–frequency distribution of each subsample matched the length–frequency distribution of all samples collected on that date. Otolith ring counts for all fish were made following the methods of Ludsin and DeVries (1997). Before calculating mean hatch dates, length-at-age regressions were computed for each site to generate ages from lengths of non-aged fish.

Individual growth through June and July was quantified using mean daily growth rates (DGR), calculated as

$$(1) \qquad \mathrm{DGR} = (\mathrm{TL}_c - 6)/A$$

where TL_c is the total length at capture, A is the total number of days from swim-up (as determined from otolith counts), and 6 mm is subtracted to correct for length obtained at swim-up (Goodgame and Miranda 1993).

To determine the relative contribution of recent versus early growth to the sizes of age-0 bass at collection, we estimated growth during the most recent 15 days. Recent growth rate (mm-day^{-1}) for the 15 days prior to capture was calculated for age-0 bass collected in July to determine growth trends close to the date of capture by measuring the width of the outer 15 otolith increments, using the following equation:

$$(2) \qquad G_{15} = [(R_2 L/M)(R_1 L/M)]/15$$

Where G_{15} is the growth during the last 15 days, R_1 is the otolith radius to the 15th increment from the outer edge, R_2 is the otolith radius to the last full increment, L is the fish length in mm, M is the maximum otolith radius along the line of measurements, and 15 is the period in days (sensu Paperno et al. 2000). Regression analyses of recent 15-day growth on age and TL indicated that growth rates were not significantly related to age ($P = 0.43$) or TL ($P = 0.32$).

Overwinter changes in juvenile largemouth bass caloric density

To evaluate the effects of first winter on age-0 bass condition, caloric densities (cal-g dry weight^{-1}) of age-0 bass collected in fall (November) were compared with caloric densities of age-1 bass collected the following spring (March). A subsample of 10 fish per site per date was selected such that it was reflective of the length distribution of the entire sample. The caloric density of each fish was determined using bomb calorimetry, as described above, except that each sample consisted of an individual bass, and replicate samples for each fish were always analyzed until two caloric densities were within 2% of one another.

Statistical procedures

We compared mean fish prey availability, energetic content of food, age-0 CPUE, hatch date, daily growth rate, and 15-day recent growth rate among sites using one-way analysis of variance (ANOVA), followed by a Tukey–Kramer adjustment for multiple comparisons ($\alpha = 0.05$) (SAS version 8.2, PROC GLM, SAS Institute Inc., Cary, North Carolina). Temporal (early summer (June–July), late summer (August), fall (October–November), and spring (March–April)) and spatial age-0 bass size trends determined from length–frequency distributions were evaluated within each site and among sites, respectively, by comparing mean TLs using the same statistical methods above. Fish prey availability and energetic content of food data were $\log_{10}$-transformed to homogenize variances.

We used logistic regression to determine the relationship between the probability of having a fish in the stomach and the total length of an age-0 bass for each site with all dates combined. Adequate models were produced by determining which parameters significantly ($P < 0.05$) explained variation in piscivory and contributed to the fit of these models' predictions to the data. Model fit was assessed using the Hosmer–Lemeshow goodness-of-fit test, which was appropriate because some largemouth bass lengths were not replicated (Kutner et al. 2004). Maximum likelihood parameter estimates were used to evaluate differences in the degree of piscivory among sites within each year, and Bonferroni-adjusted alphas were used to control for experiment error. The resulting models also were used to determine the proportion of age-0 bass that were piscivorous at 100 mm.

Results

Age-0 bass hatch dates

Although hatch dates for age-0 bass collected in June and July of 2002 and 2003 did vary slightly among sites, mean

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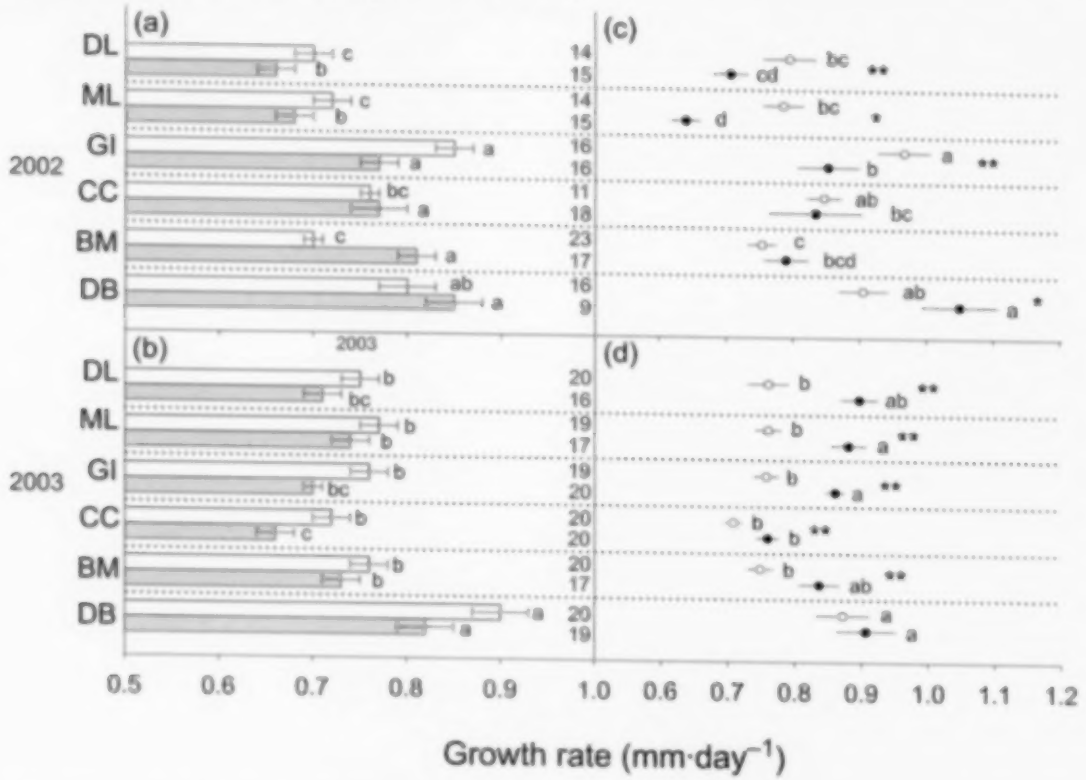
Fig. 3. Mean daily growth (MDG) rates (mm·day^{-1}, mean ± 1 standard error (SE)) for age-0 largemouth bass (*Micropterus salmoides*) collected at six sites in the Mobile–Tensaw Delta during June (shaded bars) and July (open bars) of (*a*) 2002 and (*b*) 2003: DB, D'Olive Bay; BM, Bay Minette; CC, Crab Creek; GI, Gravine Island; ML, McReynolds Lake; and DL, Dennis Lake. Sites are ordered from upstream (top, DL) to downstream (bottom, DB). The most recent 15 days of growth (mean ± 1 SE) for age-0 largemouth bass collected in (*c*) 2002 and (*d*) 2003 at each site are indicated by solid (June) and open (July) circles. Sample sizes for both MDG and 15-day recent growth (both equal for each site and date combination) are shown in panels (*a*) and (*b*). Significant differences in MDG among sites within a month and year are indicated by different letters to the right of each bar (one-way analysis of variance (ANOVA); Tukey–Kramer multiple comparison adjustment; $\alpha = 0.05$). Significant differences in recent growth among sites within a month and year are indicated by different letters to the right of each circle (one-way ANOVA; Tukey–Kramer multiple comparison adjustment; $\alpha = 0.05$). Differences in recent growth between age-0 bass collected in June and July within each site are indicated as either marginally significant (*; $0.08 > P > 0.05$) or significant (**; $P < 0.05$).



hatch dates were not correlated with mean summer growth rates across sites (Pearson's correlation coefficient; June 2002: $r = 0.17$, $P = 0.75$; July 2002: $r = 0.49$, $P = 0.33$; June 2003: $r = 0.52$, $P = 0.29$; July 2003: $r = 0.43$, $P = 0.39$) and thus were not related to growth.

First-year growth

Spring and early summer age-0 bass growth varied among sites within each year, being generally faster at sites closest to or within brackish habitats. Brackish salinities began penetrating up to Gravine Island (GI) during early summer 2002, but in 2003 did not reach any study sites until August (Fig. 2). In June 2002, age-0 bass present at brackish sites (i.e., D'Olive Bay, DB; Bay Minette, BM; CC; and GI) exhibited higher mean daily growth rates than those at freshwater sites (i.e., McReynolds Lake, ML; and DL) (Fig. 3*a*; Tukey–Kramer, all $P < 0.05$). Samples from July 2002 showed increased mean daily growth at GI (one-way ANOVA, $F_{[1,30]} = 8.82$, $P = 0.006$) and no change at DB ($F_{[1,23]} = 0.79$, $P = 0.38$), but still faster growth than those at ML and DL (Fig. 3*a*; Tukey–Kramer; all $P < 0.05$). Fifteen-day recent growth trends showed similar patterns to mean daily growth (Fig. 3*c*), but only age-0 bass at ML showed a highly significant increase from June to July ($F_{[1,27]} = 8.24$, $P = 0.005$). Age-0 bass at two other upstream sites (i.e., GI ($F_{[1,27]} = 3.83$, $P = 0.06$) and DL ($F_{[1,27]} = 3.46$, $P = 0.07$)), however, did show a marginally significant increase during the same period, and those at DB showed a significant ($F_{[1,23]} = 23.60$; $P < 0.001$) decrease during a period of increasing salinity at this site.

By August 2002, age-0 bass at DB still appeared to exhibit the fastest growth, as their mean total lengths were longer than age-0 bass at all other sites except GI (Fig. 4). Age-0 bass at ML were smallest, but were only significantly smaller than those at DB and GI (Tukey–Kramer; $P < 0.0001$). Age-0 bass at all sites appeared to continue to grow

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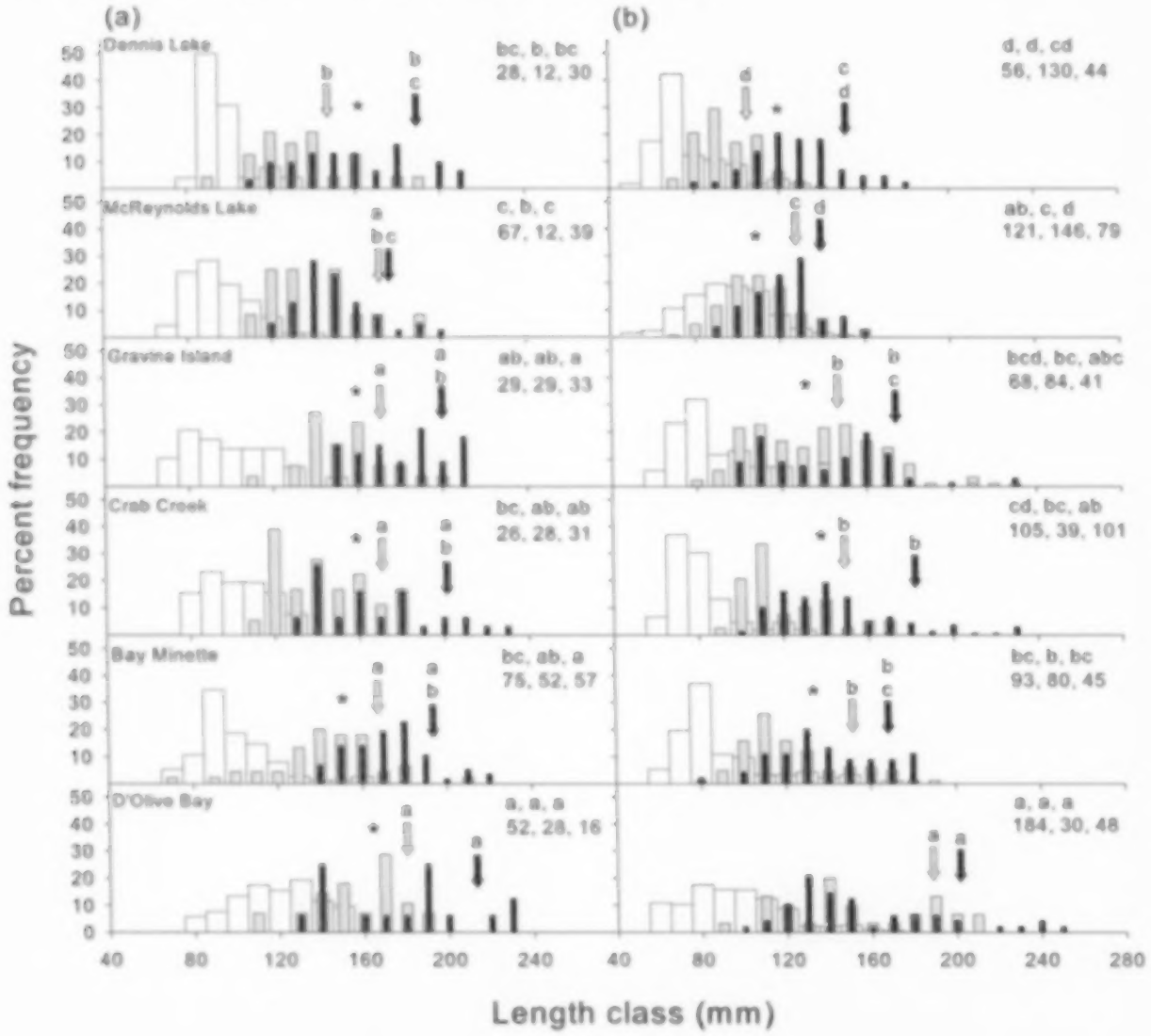
Fig. 4. Late summer (open bars), fall (shaded bars), and spring (solid bars) length–frequency distributions for the (*a*) 2002 and (*b*) 2003 age-0 largemouth bass (*Micropterus salmoides*) year classes collected at six sites in the Mobile–Tensaw Delta, Alabama. Differences in mean total length (TL) among sites within each season and year are indicated by different letters in order of season (i.e., late summer, fall, spring) in the upper right-hand corner of each histogram (one-way analysis of variance (ANOVA); Tukey–Kramer; $\alpha = 0.05$). Sample sizes for each site, within each season and year are also listed in order of season (i.e., late summer, fall, spring) in the upper right-hand corner of each histogram. Significant differences in mean TL between fall- and spring-collected age-0 largemouth bass within each site are indicated with an asterisk (*) (one-way ANOVA). Upper quartile mean TLs for fall (shaded) and spring (solid) are indicated with arrows. Differences in upper quartile mean TLs among sites within each season and year are indicated by different letters above the respective arrows (one-way ANOVA; Tukey–Kramer; $\alpha = 0.05$).



into the fall, as lengths were significantly longer in the fall compared with August (all $P < 0.05$). In addition, growth patterns exhibited among sites during summer continued into fall, as age-0 bass at DB still exhibited the largest size, and were significantly larger than those at ML ($P = 0.03$) and DL ($P = 0.004$). The propensity for faster growth at brackish sites (i.e., DB, BM, CC, GI) also was indicated by the presence of larger upper quartile mean TL at these sites than at those at DL.

During winter, age-0 bass continued to grow at all sites except ML, as mean lengths were significantly longer in spring compared with the previous fall (Fig. 4). In fact, growth patterns present in the summer appeared to carry through until spring, generally producing the largest age-1

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Table 1. Mean caloric density (cal·g dry weight^{-1}, mean ± 1 standard error (SE)) of the 2002 and 2003 year classes of largemouth bass (*Micropterus salmoides*) collected during their first fall and subsequent spring (i.e., age-1) in the Mobile–Tensaw Delta, Alabama, USA.

	2002 year class		2003 year class	
Site	Fall 2002	Spring 2003	Fall 2003	Spring 2004
Dennis Lake	4108.8±23.9*	4540.6±40.5*	4273.6±73.9	4222.3±48.2
McReynolds Lake	4217.9±50.9	4380.7±59.9	4063.8±69.7	4146.1±45.4
Gravine Island	4077.8±48.7	4104.4±49.7	4257.3±36.8	4265.0±30.3
Crab Creek	4094.5±28.5	3997.9±51.5	4355.6±61.4*	4073.2±21.3*
Bay Minette	4212.4±31.9	4136.3±66.9	4289.4±61.8	4192.6±38.7
D'Olive Bay	4100.7±25.3	4070.4±40.5	4197.6±73.9	4359.1±48.2

Note: Significant differences within a year class and between fall and spring collections within a site are indicated by an asterisk (*) (one-way analysis of variance, $\alpha = 0.05$). $n = 10$ for all sites.

fish at downstream sites. Age-1 bass at GI, DB, and BM were the longest and were significantly longer than those at ML and DL (all $P < 0.02$). Age-1 bass at CC also were significantly longer than those at ML (both $P < 0.01$).

Although no sites were brackish during summer 2003, mean daily growth rates of age-0 bass collected in June and July showed that growth was fastest at DB (Fig. 3*b*; one-way ANOVA; June: $F_{[5,103]} = 8.76$, $P < 0.0001$; July: $F_{[5,112]} = 7.35$, $P < 0.0001$; Tukey–Kramer, all $P < 0.05$). Comparison of growth rates for age-0 bass collected in June versus July showed increases at DB ($F_{[1,37]} = 3.52$, $P = 0.07$), CC ($F_{[1,38]} = 5.56$, $P = 0.02$), and GI ($F_{[1,37]} = 8.39$, $P = 0.006$), but no change at all other sites. Fifteen-day recent growth rates indicated that age-0 bass were growing at relatively similar rates in June; however, by July those at DB were growing significantly faster (Fig. 3*d*; $F_{[5,112]} = 4.73$, $P = 0.0006$; Tukey–Kramer, all $P < 0.05$). In addition, recent growth trends showed that age-0 bass at all sites except DB were growing slower in July than in June (Fig. 3*d*).

In 2003, the size patterns observed in early summer were also present in August, with age-0 bass at DB remaining the largest among all sites, except those at ML (one-way ANOVA, $F_{[5,62]} = 12.87$, $P < 0.0001$; Tukey–Kramer, all $P < 0.05$). From late summer to fall 2003, age-0 bass at all sites exhibited increases in length, suggesting growth during this period (all $P < 0.0001$). During fall, age-0 bass at DB were still the longest, and those at DL were the smallest (all $P < 0.05$). At this point, a brackish environment was present up to GI (Fig. 2) and appeared to confer a growth advantage for some of these age-0 bass, as those at BM were now significantly longer than those at ML and DL ($P = 0.0002$), and those at CC and GI were longer than those at DL ($P < 0.0001$). The propensity for faster growth at brackish sites (i.e., DB, BM, CC, and GI) also was shown by age-0 bass upper quartile lengths, which were larger than at ML and DL (all $P < 0.05$) (Fig. 4).

Overwinter changes in length–frequency distributions were again apparent in 2003 (Fig. 4). Age-1 bass collected in spring 2004 were significantly longer than fall-caught age-0 bass at all sites except DB. Like spring 2003, age-1 sizes in spring 2004 indicated a growth and size advantage at downstream sites, as age-1 bass at DB, BM, CC, and GI were larger than those at ML, and those at DB also were larger than those at DL (all $P < 0.05$; Fig 4). Upper quartile lengths further substantiated this advantage, as large age-1 bass at DB were longer than those at all other sites ($P < 0.05$; Fig. 4). In addition, large age-1 bass at CC were longer than those at ML and DL, while those at GI and BM were longer than those at ML (all $P < 0.05$; Fig. 4).

Overwinter temperature and changes in largemouth bass caloric density

Largemouth bass caloric densities generally showed little change through the winter of either year. Overwinter changes were only observed at DL during the 2002–2003 winter ($F_{[1,18]}$, $P < 0.0001$) and at CC during the 2003–2004 winter ($F_{[1,18]}$, $P = 0.0004$; Table 1). During the 2002–2003 winter, continuous temperature data were only available at CC and DL, where temperatures dropped below 10 °C for 66 days (19 December – 24 February) and 31 days (4 January – 3 February), respectively. Temperatures were <6 °C for three days in January 2003 at CC, but never were <6 °C at DL. Caloric densities were not depressed at any site through the 2002 winter and even showed significant increase at DL (one-way ANOVA; $F_{[1,18]} = 38.29$, $P < 0.0001$; Table 1). During the 2003–2004 winter, continuous temperature data were again only available at CC and DL, where temperatures were never <10 °C for any extended period, but fluctuated between 8 and 11 °C for 76 days (15 December – 29 February). No temperatures <6 °C were observed at either site. In 2003, caloric densities again remained stable through the winter at all but one site, with bass at CC exhibiting a significant decline ($F_{[1,18]} = 18.89$; $P = 0.0004$; Table 1).

Age-1 recruitment

No significant differences in age-1 relative abundance (catch·10 min^{-1}) were observed in spring 2003 (DB: 4.9 ± 0.9; BM: 3.7 ± 0.6; CC: 8.4 ± 1.3; GI: 3.6 ± 0.8; ML: 7.6 ± 3.5; DL: 4.3 ± 2.2; one-way ANOVA; $F_{[5,57]} = 1.49$, $P = 0.21$) or 2004 (DB: 2.6 ± 0.9; BM: 8.6 ± 2.8; CC: 3.9 ± 1.2; GI: 4.7 ± 1.8; ML: 5.7 ± 1.7; DL: 3.9 ± 0.7; $F_{[5,48]} = 1.60$, $P = 0.18$).

Diet composition, energetic content of food consumed, and transition to piscivory

Energetic content of food consumed by age-0 bass at DB was consistently highest during early summer of both years (Fig. 5). In 2002, age-0 bass at DB had significantly higher energetic content of food consumed during early summer than at all other sites (one-way ANOVA, $F_{[5,456]} = 14.42$, $P <$

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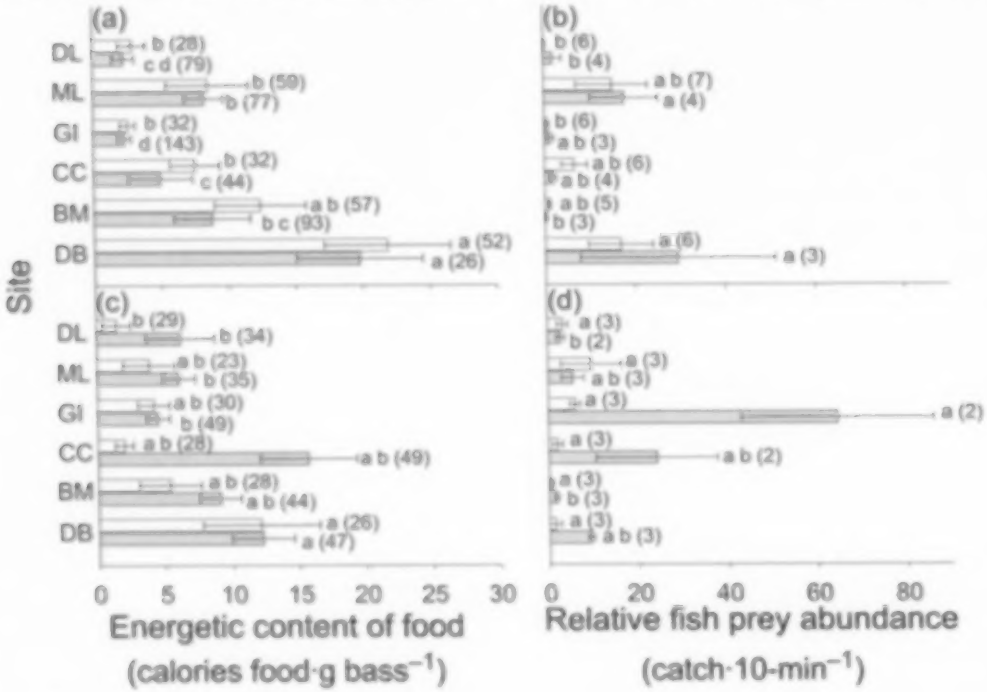
Fig. 5. Mean energetic content of food consumed (cal food·g bass^{-1} ± 1 standard error (SE)) by age-0 largemouth bass (*Micropterus salmoides*) at six sites in the Mobile–Tensaw Delta, Alabama, during (*a*, *b*) early summer (June–July) and (*c*, *d*) late summer (August) in 2002 (shaded bars) and 2003 (open bars): DB, D'Olive Bay; BM, Bay Minette; CC, Crab Creek; GI, Gravine Island; ML, McReynolds Lake; and DL, Dennis Lake. Differences among sites within a season and year are indicated by different letters (one-way analysis of variance (ANOVA); log-transformed (x + 1) data; Tukey–Kramer; α = 0.05). Sample sizes are in parentheses next to letters. Relative fish prey abundance (catch·10 min transect^{-1}) of age-0 largemouth bass collected during early summer and late summer 2002 (shaded bars) and 2003 (open bars). Differences in fish prey abundance among sites within a season are indicated by different letters (one-way ANOVA; log-transformed (x + 1) data; Tukey–Kramer; α = 0.05). The number of transects are in parentheses next to the letters.



0.0001; Tukey–Kramer, all $P < 0.05$), and in 2003 only age-0 bass at BM (12.42 ± 3.42 cal food·g bass^{-1}) had similar energetic content of food consumed (Fig. 5; $P = 0.32$). Age-0 bass at all sites upstream of DB generally exhibited similar energetic content of food consumed within each year (Fig. 5).

During late summer of both years, energetic content of food consumed again was high at DB; however, only age-0 bass at DL had fewer calories in both years (Fig. 5; 2002: $F_{[5,292]} = 3.93$, $P = 0.002$; 2003: $F_{[5,150]} = 5.02$, $P = 0.003$; Tukey–Kramer, both $P < 0.05$). All other age-0 bass upstream of DB had similar energetic content of food consumed during both years, except those at GI, ML, and DL, which had lower energetic content of food in 2002 (Fig. 5; all $P < 0.05$).

The onset of piscivory by size or date did not appear to vary strongly among sites during either year, and neither size nor date of onset was related to growth patterns (Pearson's correlation coefficient; size of onset vs. mean daily growth: $r = 0.31$, $P = 0.33$; time of onset vs. mean daily growth: $r = 0.37$, $P = 0.24$).

The proportion of piscivorous fish did exhibit a logistic increase with age-0 bass length at all sites in both 2002 and 2003 (Fig. 6). In 2002, the degree of piscivory-at-length was greatest at DB, ML, and CC. Age-0 bass at DB and ML showed significantly greater piscivory-at-length than those at BM, GI, and DL (Hosmer–Lemeshow: $\chi^2 = 12.49$, $P = 0.13$; main effect of site, Wald: $\chi^2 = 51.83$, $P < 0.0001$; all Bonferroni-adjusted contrasts $P < 0.003$; Fig. 6). This model also indicated that 68% and 71% of age-0 bass at DB and ML, respectively, were piscivorous at 100 mm in comparison with 38%, 50%, 19%, and 30% at BM, CC, GI, and DL, respectively.

In 2003, DB exhibited a significantly greater proportion of piscivorous age-0 bass compared with all other sites (Hosmer–Lemeshow: $\chi^2 = 5.02$, $P = 0.76$; Wald: $\chi^2 = 46.12$, $P < 0.0001$; all $P < 0.003$). In addition, 51% of age-0 bass at DB were piscivorous at 100 mm compared with 22%, 17%, 12%, 22%, and 15% at BM, CC, GI, ML, and DL, respectively.

Relative fish prey availability

Relative fish prey abundance was consistently higher at DB than at BM and DL during early summer of both years (Fig. 5). ML also had more fish prey available than BM and DL in the early summer of 2002 (one-way ANOVA, $F_{[5,15]} = 6.96$, $P = 0.0015$; Tukey–Kramer, all $P < 0.02$), but not in 2003 (Fig. 5). By proportion, the species found in our sam-

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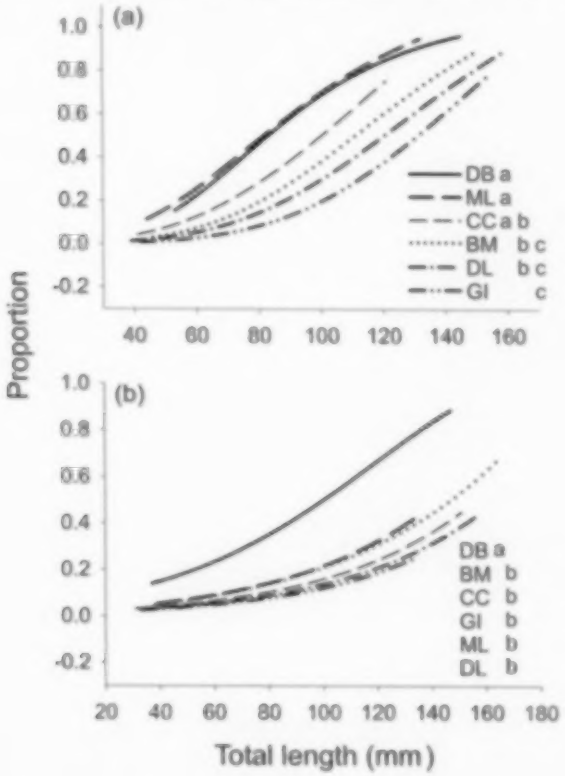
Fig. 6. Predicted relationships between total length (TL, mm) and the probability of age-0 largemouth bass (*Micropterus salmoides*) having fish in the diet (i.e., piscivorous) for fish collected at six sites in the Mobile–Tensaw Delta during summer (*a*) 2002 (maximum likelihood logistic regression; Hosmer–Lemeshow goodness of fit: $\chi^2 = 12.49$, $P = 0.13$; main effect of site, Wald: $\chi^2 = 51.83$, $P < 0.0001$; DB, $n = 107$; BM, $n = 113$; CC, $n = 114$; GI, $n = 126$; ML, $n = 86$; DL, $n = 97$) and (*b*) 2003 (Hosmer–Lemeshow goodness of fit: $\chi^2 = 5.02$, $P = 0.76$; Wald: $\chi^2 = 46.12$, $P < 0.0001$; DB, $n = 66$; BM, $n = 117$; CC, $n = 59$; GI, $n = 118$; ML, $n = 105$; DL, $n = 80$). DB, D'Olive Bay; BM, Bay Minette; CC, Crab Creek; GI, Gravine Island; ML, McReynolds Lake; and DL, Dennis Lake. Site-specific line symbols in panel (*b*) are the same as those represented in panel (*a*). Significant differences in the degree of piscivory among sites within each summer are indicated by different letters next to site abbreviations in each panel (pairwise maximum likelihood contrasts; Bonferroni-adjusted $\alpha = 0.003$).



ples varied considerably among sites during the summer. In 2002, rainwater killifish (*Lucania parva*) were dominant at DB (96%, $n = 130$) and common at CC (38%, $n = 7$). Bay anchovy (*Anchoa mitchilli*) were common at both GI (64%, $n = 29$) and DL (85%, $n = 69$), and *Fundulus* spp. (38%, $n = 59$) and western mosquitofish (*Gambusia affinis*) (31%, $n = 48$) were common at ML. Only one western mosquitofish of available size was collected at BM during summer 2002. Rainwater killifish were again common at DB (26%, $n = 26$) in 2003, but Gulf menhaden (*Brevoortia patronus*) were more abundant (64%, $n = 65$). Rainwater killifish also were common at BM (83%, $n = 5$) and western mosquitofish were common at CC (81%, $n = 29$) and GI (48%, $n = 2$), although only two fish of available size were collected at GI. Inland silverside (*Menedia beryllina*) were abundant at ML (62%, $n = 46$), and only one fish was found to be available at DL.

During late summer, GI had more fish prey available than BM (one-way ANOVA, $F_{[5,91]} = 6.08$, $P = 0.0099$; Tukey–Kramer, $P = 0.007$) and DL ($P = 0.03$) in 2002; however, no other differences were observed among sites within each year (Fig. 5).

Discussion

Factors influencing spring and summer growth

Age-0 largemouth bass exhibited strong spatial variability in growth and thus length within the Delta. During both years, mean daily growth rates of fish collected in June (and in July 2003) were significantly greater at sites either in or adjacent to brackish water habitats. Recent growth rates showed similar patterns, indicating that this growth trend was present during the month of capture, although it may have initiated in spring. In addition, growth rates were consistently higher at DB (downstream) than at ML and DL (both upstream), where there was no measurable salinity throughout the summers of 2002 and 2003. From DB to GI, the 2002 year class was exposed to oligohaline to mesohaline conditions from March through November, and in 2003 freshwater conditions were present until mid-August, when oligohaline characteristics emerged at the same sites. This was in contrast with ML and DL, which were consistently fresh water during both years. Estuarine-dependent and resident species also have been shown to exhibit faster growth in oligohaline and mesohaline conditions relative to fresh water (Altinok and Grizzle 2001); however, these growth patterns have not been documented for a freshwater species in a brackish environment. In fact, most research corroborates expected physiological responses of freshwater species (but see Musselman et al. 1995), with higher growth in freshwater compared with oligohaline or mesohaline environments (Peterson 1991; Altinok and Grizzle 2001). Although age-0 bass at DB, BM, CC, and GI did not appear to show a negative response to salinity, recent daily growth patterns indicated slower growth at DB from June to July 2002 and no change in growth rate at BM and CC during the same period. However, of those age-0 bass upstream from CC, where salinity was always <1 ppt, all showed an increase in growth during the same period. These results suggest increased energetic costs associated with exposure to higher salinities at DB and potentially at BM and CC as well. In the laboratory, juvenile largemouth bass can tolerate salinity up to 10 ppt by increasing metabolic rates while maintaining osmotic pressure within the range observed while in fresh water (Susanto and Peterson 1996). However, Susanto and Peterson (1996) also showed that oxygen consumption was significantly higher at 4 ppt and 12 ppt than at 0 ppt. The fact that fish at DB were exposed to salinities up to 12 ppt and possibly higher (our data were not continually recorded, but collected at monthly intervals) suggests that these fish had to make energetic and possibly behavioral (i.e., movement to lower salinity portions of the local habitat) compromises during summer 2002.

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Despite the apparent negative effects of salinity, age-0 bass at DB maintained larger sizes than those at BM, CC, ML, and DL throughout summer 2002. Although this pattern may have emerged in the spring, the continued presence of this pattern into June, as shown by recent growth rates, appeared to be attributable to higher energetic content of food consumed by age-0 bass at DB than at all other sites from June through July. Similar recent growth patterns in June at all other sites that experienced salinity may also have been because of high energetic content of food. Although energetic content of food was not statistically different at these sites compared with ML and DL where slower-growing age-0 bass were present, the levels at BM and CC showed a tendency to be higher than those at DL, and thus these sites may have provided a biologically significant resource advantage.

Food quality and quantity is considered a critical limiting factor in growth of largemouth bass, and lack of suitable prey at either the insectivorous or piscivorous feeding stages reduces age-0 bass growth rates (Aggus and Elliott 1975; Keast and Eadie 1985). Although available prey showed high variation among replicate samples at DB, there was clearly a tendency for higher prey fish production at this site than at all other sites except ML during early summer. This trend appears to be due to abundant small fish prey at this site, although larger size age-0 bass also likely contributed to this trend. Regardless, high prey fish availability enabled greater piscivory at DB and likely permitted a continued rapid growth response despite the probable metabolic stresses caused by salinity. These observations highlight a potential benefit of these low-salinity habitats that has not been observed before for largemouth bass or other freshwater species found in natural brackish water environments. Our results are also in contrast with those observed for adult coastal largemouth bass, which have been observed to consume more fish and grow faster in freshwater compared with brackish water habitats despite apparently high fish prey abundance in brackish habitats (Meador and Kelso 1990). Meador and Kelso (1990) suggested that the discrepancy between fish prey abundance and the degree of piscivory in brackish habitats was due to predation pressure by alligators (*Alligator mississippiensis*), alligator gar (*Atractosteus spatula*), and red drum (*Sciaenops ocellatus*), which forced adults to forage less efficiently in vegetated habitats. Although the age-0 bass in this study were also found predominantly in vegetation, their small size likely enabled them to forage more efficiently than adults under such conditions.

Unlike freshwater age-0 bass in the southeastern USA, which often eat juvenile shad (primarily *Dorosoma* spp.) or sunfishes (*Lepomis* spp.), age-0 bass at DB, the most brackish water site, were primarily consuming rainwater killifish (Peer 2004), a small resident species commonly found in coastal brackish habitats (Mettee et al. 1996). In freshwater systems, the threshold size for a shift from feeding on invertebrates to feeding on fish increases through the season, largely because of progressive increases in prey length (Olson 1996). The small maximum size of rainwater killifish (50 mm) and their production of young between April and August (Kuntz 1916; Hilderbrand and Schroeder 1928), however, may substantially reduce the threshold size for piscivory and increase fish prey availability to a wider range of sizes throughout the summer. Small maximum size also might have prevented rainwater killifish from outgrowing the gape of largemouth bass once largemouth bass reach 100 mm. In fact, once largemouth bass reached 100 mm in DB, approximately 68% were piscivorous in 2002, while 51% were piscivorous in 2003. The link between this abundant estuarine prey resource and more rapid growth of age-0 bass at this site suggests a potential benefit of low-salinity habitats, possibly enabling a freshwater fish to not only overcome the metabolic costs of living in such a stressful environment, but also retain sufficient reserves to grow at a rate comparable with fast-growing freshwater largemouth bass in other southern USA systems (e.g., Ludsin 1994). In general, however, the higher energetic content of food consumed by age-0 bass at DB likely was due to greater consumption of fish, which in turn fueled more rapid growth. Relatively rapid growth at BM, CC, and GI, however, could not be explained as clearly by these same processes, as energetic content of food, fish prey availability, and the proportion of piscivorous fish were all statistically lower at these sites than at DB and similar to other sites. This may have been due to lower salinity at BM, CC, and GI during June and July, which may have resulted in lower energetic costs than at the two freshwater sites. As previously mentioned, Sasanto and Peterson (1996) showed increased oxygen consumption with increasing salinity, although they did not measure oxygen consumption between 0.1 ppt and 4 ppt, which is within the range of salinities observed at these sites in June and July. In fact, it has been suggested that metabolic rate should decline in freshwater fish as environmental osmolarity increases to near isotonicity (Igram and Wares 1979; Furspan et al. 1984). While salinities at these sites were not near isotonicity for largemouth bass (~8 ppt), reduced metabolism may still have provided a growth advantage at very low salinities.

In 2003, when freshwater conditions were present through the end of the summer, age-0 bass at DB again exhibited fast growth. Although an initial rapid growth response in spring may have permitted the high mean daily growth rate observed at DB, recent daily growth trends from age-0 bass collected in June and July indicated that rapid growth at DB continued into the summer. As during summer 2002, faster growth during summer appeared to again be due to higher energetic content of food consumed, resulting from a greater proportion of fish in the diet, afforded by increased fish prey availability in the form of rainwater killifish. This trend suggests that salinity per se may not be a key correlate in explaining the apparent benefits of downstream habitats. Instead, a more critical factor may simply be proximity to brackish habitats or the intermittent intrusion of saline waters, which maintain a brackish community in habitats that may frequently exhibit the chemical properties of fresh water.

Transition to piscivory

For piscivores, the shift from an invertebrate to primarily a fish diet is critically important to early growth (Keast and Eadie 1985; Mittelbach and Persson 1998). This is because a fish diet is superior to an invertebrate diet in gross energy intake and capture efficiency (Werner and Gilliam 1984;

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Mittelbach and Persson 1998). Consequently, the switch to piscivory often initiates an increase in growth (Aggus and Elliott 1975; Post et al. 1998). In most systems, it is important for largemouth bass to make this shift in the first summer to maintain a size advantage over their prey so that piscivory can continue (e.g., Miller and Storck 1984; Madenjian and Carpenter 1991; Olson 1996). In addition, large prey size may reduce predation risk through enhanced swimming ability (Christensen 1996) and reduced vulnerability to gape-limited predators (Hambright 1991).

In this study, the shift to piscivory was variable among cohorts from different sites with similar hatch dates, yet summer growth rates did not correlate with the time or size of initial transition to piscivory. Largemouth bass at DB grew the fastest, yet these age-0 bass did not begin consuming fish at the earliest time or at the smallest size. In fact, largemouth bass at ML, which exhibited significantly slower growth in 2003, transitioned earlier and at a smaller size. The lack of concordance between growth and the transition to piscivory indicates other factors, such as the degree of piscivory, may be more important when assessing growth differences among cohorts from distinct subpopulations.

Because unique processes and interactions occurred at each site in the Delta, timing or size at piscivory at one site might not afford the same advantage as similar timing or size at piscivory at another site. Likewise, as was observed in this study, early timing or smaller size at piscivory might not translate into faster growth. Most studies that suggested the transition to piscivory as a critical period assessed one population (Shelton et al. 1979; Post 2003) or several populations under similar conditions (Olson 1996; Ludsin and DeVries 1997) and usually noted an advantage to early-spawned fish. In theory, for the onset of piscivory to operate as a critical period, certain individuals must be at an advantage (usually larger and (or) older), piscivory must be maintained, and the advantage of shifting to piscivory should be the same for all individuals. If one of these terms is not met, the advantage of the transition to piscivory may not be assured.

In the Delta, spatial segregation of subpopulations and the unique ecological processes operating at each site caused the transition to piscivory to have a differential effect for individuals at each site. The disconnection between transition to piscivory and growth was most likely due to site-specific abiotic or biotic factors influencing availability of fish prey and (or) fish growth conditions at each site. Low or inconsistent prey availability appeared to delay or prevent transition to a mostly piscivorous diet for age-0 bass exhibiting slower growth and smaller sizes than those bass at DB. This delay was most apparent at DL in 2002 and at all sites upstream of DB in 2003. Consequently, even if age-0 bass at one site started consuming fish at an earlier date or smaller size than those at DB, the advantage was never realized because a diet dominated by fish could not be maintained at the same level exhibited by bass at DB. Although age-0 bass at CC and ML did show a similar transition, those at ML grew slower and remained smaller throughout the summer. Age-0 bass at ML also had lower calories consumed during early and late summer, indicating that although they were consistently eating fish, they were not eating as many as those at DB. As a result, the degree of piscivory, as indicated by the consistency of piscivorous feeding, as well as number, mass, or calories of fish eaten had greater influence on growth than the mere transition to piscivory.

Fall and first winter growth and condition

During this study, growth continued through fall at all sites during both years. Fall 2002 size patterns were generally consistent with those observed during summer 2002; however, in 2003, size patterns indicated improved growth through November at those sites that became brackish during fall (i.e., BM, CC, GI) relative to sites that remained fresh (i.e., ML and DL). Summer growth and fall size have frequently been implicated as important determinants of overwinter survival and recruitment success (Post and Evans 1989; Cargnelli and Gross 1996; Ludsin and DeVries 1997). Size-dependent winter survival is common in southern USA largemouth bass populations (Aggus and Elliott 1975; Adams et al. 1982; Miranda and Hubbard 1994*a*), but less evidence of this phenomenon exists for northern populations (e.g., Kohler et al. 1993; Wright 1993; Garvey et al. 1998). This is despite the implication that overwinter mortality is often due to starvation caused by reduced feeding at temperatures <6 °C (Garvey et al. 1998). Although water temperatures can drop below 6 °C in many southern systems, Ludsin and DeVries (1997) suggested that metabolic costs associated with fluctuating winter water temperatures might limit this phenomenon to southern latitudes.

Size-selective overwinter mortality was not detected at any of our sampling sites, and higher recruitment did not occur at sites with larger fall age-0 bass in the 2 years of our study. Jackson and Noble (2000) found similar results in a southern USA reservoir (Jordan Lake, North Carolina). Largemouth bass caloric densities also did not decline over winter, except at CC during winter 2003–2004. This observation indicates that energy reserves were not being depleted during winter. Larger mean fish length in spring than in fall also suggests that consumption exceeded maintenance requirements. As noted by Jackson and Noble (2000), if strong size-selective overwinter mortality had been acting on age-0 bass, its effects would probably have severely truncated age-1 length–frequencies. Length–frequencies in the Delta, however, were not truncated at any site, but did shift to the right, indicating growth during the winter.

The results of this study, combined with that of Jackson and Noble (2000) and others that have experimentally explored size-dependent overwinter mortality in age-0 bass (Miranda and Hubbard 1994*b*; Ludsin and DeVries 1997; Garvey et al. 1998), suggest that size-selective overwinter mortality is the result of a complex interaction of winter severity, food availability, and predation. When winter water temperature falls below 6 °C for extended periods, small individuals experience greater proportional energy loss and increased mortality relative to large individuals (Garvey et al. 1998). However, at southern latitudes with shorter, warmer winters, juvenile largemouth bass may continue to grow if food is available. Small members of a cohort may still experience higher mortality at these low latitudes because of the direct effect of size-selective predation and the effects of starvation (which can occur when food is not available in the

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correct size range and when avoiding predation places them in habitats with little food or stressful conditions). These effects may be intensified at southern latitudes because of higher metabolic rates and greater energetic requirements of both age-0 bass and their predators (Miranda and Hubbard 1994*b*; Garvey et al. 1998). Warm or fluctuating water temperature also may prevent small individuals from developing physiological conditions such as dormancy that reduce energy expenditure over long cold winters (Crawshaw 1984; Wright et al. 1999). Because of the complex interactions among physiological, behavioral, and habitat conditions, size-selective overwinter mortality of age-0 bass in southern latitudes is likely to be highly variable among systems and across years within systems. During 2002 and 2003 in the Mobile–Tensaw Delta, winter conditions favored the survival of all size classes of age-0 bass, food was relatively abundant, and predation risk was apparently low. While potential predators, particularly adult largemouth bass, are abundant in the Delta, vegetation (both submersed and emergent) that remained during winter may have provided refuge for juvenile largemouth bass while abundant alternative prey for bass predators also was available (A.C. Peer, personal observation).

Recruitment and age-1 size

Hatch date (Isely and Noble 1987; Phillips et al. 1995), transition to piscivory (Aggus and Elliott 1975; Ludsin and DeVries 1997), fall lipid accumulation (Sullivan 1986; Miranda and Hubbard 1994*a*, 1994*b*), and overwinter survival (Isely 1981; Miranda and Hubbard 1994*a*, 1994*b*), either together (Ludsin and DeVries 1997) or independently, have been considered critical periods that influence largemouth bass recruitment to age-1. In this study, hatch date, transition to piscivory, fall caloric density (a surrogate for lipids), and overwinter survival were similar among sites and generally led to similar age-1 abundances in the spring. This is despite the fact that each of these independent critical periods did not operate as predicted. In other words, fish that grew faster did not hatch earlier nor did they show earlier onset of piscivory. Additionally, larger fish did not have higher caloric densities in the fall or greater overwinter survival, but did have similar recruitment.

Despite an apparent similarity in recruitment among sites, sizes were different and generally reflected the same size patterns present in the summer. During both years, largemouth bass located in brackish or near-brackish habitats were either the largest or among the largest in the Delta. This was particularly evident at DB, the site located furthest downstream, and thus most likely to be influenced by brackish conditions, even when its physicochemical conditions resembled fresh water. These results suggest a growth benefit of these environments to young largemouth bass, which is likely attributed to prey availability during summer and probably throughout the year, as young-of-the-year estuarine-dependent fish (e.g., Gulf menhaden, Atlantic croaker (*Micropogonias undulates*), and spot (*Leiostomus xanthurus*)) enter the lower Delta in winter and early spring following offshore fall spawning. Although this suggestion is fairly well supported at DB during both years, other factors such as reduced osmoregulatory energy expenditures or interspecific competition may have enabled fast grow at BM, CC, or GI in 2002. Nevertheless, larger age-1 fish do potentially have a fitness benefit that is frequently ignored when considering largemouth bass recruitment (but see Post 2003). Often the effects on early life are forgotten once largemouth bass reach age-1, despite the potential for early life processes to have important continuous implications for future recruitment. Larger fish typically exhibit greater fecundity (Wootton 1990) and earlier maturity (Baylis and Wiegmann 1993) and consequently should have greater lifetime fecundity (Post 2003). However, in addition to greater individual fitness, larger juveniles could potentially have a greater influence on future population dynamics through greater reproduction and simultaneously greater genetic contribution. If habitats differ in the size of individuals produced and thus the overall fecundity, then habitats that produce larger juvenile largemouth bass may serve a function similar to sources in a metapopulation by contributing disproportionately to future adult recruitment. However, unlike classic metapopulation theory where juveniles act as the dispersers, here adults and older juveniles are the more likely dispersers, given the rather sessile nature of age-0 bass.

Together, these spatial recruitment and growth trends suggest a nursery benefit of low-salinity brackish or near-brackish environments to age-0 bass. Because age-0 bass growth rates are affected by natural variability in environmental factors, they can be good predictors of habitat suitability (Sogard 1992; Brandt and Kirsch 1993). Not only do these habitats appear to provide sufficient food resources, but they also appear to be located sufficiently downstream so as to experience less impact from freshwater pulses because of flow reduction associated with shallower depths and greater surface area of water downstream.

In conclusion, while our results do not discount the importance of critical periods for largemouth bass, this work does identify the importance of factors not necessarily thought to be important previously, albeit in a strikingly different system. Most notably, the degree of piscivory, rather than the actual transition to piscivory, was more important in this system; the initial transition to piscivory did not necessarily translate into the continuation of piscivory, nor did it account for the amount of fish eaten. Smaller fish prey (e.g., rainwater killifish) than those that are typically abundant in purely freshwater systems also appeared to offer age-0 bass in brackish habitats a resource that is broadly available to a wide size range of largemouth bass.

These findings also support the need to incorporate spatial and temporal variability when studying recruitment and growth in such heterogeneous systems. As shown here, age-0 largemouth bass will exhibit habitat-dependent growth that can be either rapid or marginal. Because the proportions of particular habitat types within a system can determine future population dynamics and because these factors may influence adult fish size, high-quality nursery habitats and environmental conditions should be identified. In this study, age-0 bass benefited from low-salinity coastal environments and those closest to such environments, as opposed to the habitats that were consistently fresh water. Although this pattern was robust given the strong variation in hydrologic and physicochemical conditions

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between the 2 years of this study, further work will be necessary to confirm this pattern in other coastal systems. However, if these patterns hold, protection of productive habitats during spawning may provide an additional management strategy to improve recruitment where necessary, as well as to enhance juvenile growth and potentially adult largemouth bass growth.

Although several habitat conditions important to growth were identified, additional experimental work is required to isolate causal factors responsible for differential growth observed among subpopulations. This knowledge, when combined with work on the implications of early growth to eventual fecundity and recruitment, will enhance our ability to predict future population dynamics, allowing improved conservation and management of habitats and subpopulations that may contribute disproportionately to the population.

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The catchability of large American lobsters (*Homarus americanus*) from diving and trapping studies off Grand Manan Island, Canadian Maritimes

M.J. Tremblay, S.J. Smith, D.A. Robichaud, and P. Lawton

Abstract: Catchability (q) in traps was estimated for American lobsters (*Homarus americanus*) in Flagg Cove, off Grand Manan Island (New Brunswick, Canada), where large females (>100 mm carapace length (CL)) aggregate in late summer and early fall. In 2001 and 2002, diver surveys were used to estimate lobster density, and traps were then deployed to obtain catch rates. Bayesian generalized linear models were fit to the densities of different size groups (81–100, 101–130, 131–160, and >160 mm CL) of ovigerous females, non-ovigerous females, and males. Catchability was strongly affected by year. Differences in q due to sex and size for ovigerous females, non-ovigerous females, and males were apparent but were not consistent between years. Size was not an important factor for the catchability of lobsters between 81 and 160 mm CL. In comparison with lobsters of a similar size in other areas, Flagg Cove lobsters in the size range of 81–100 mm CL were less catchable. We hypothesize that this resulted from the high densities and larger sizes of lobsters in Flagg Cove, which likely lead to increased agonistic interactions and reduced entry of lobsters into traps.

Résumé : Nous avons estimé la capturabilité (q) des homards américains (*Homarus americanus*) dans les casiers à Flagg Cove, au large de l'île du Grand Manan (Nouveau-Brunswick, Canada), où les femelles de grande taille (>100 mm de longueur de carapace, CL) se rassemblent à la fin de l'été et au début de l'automne. En 2001 et 2002, des inventaires par des plongeurs ont permis d'estimer la densité des homards; des casiers ont ensuite été placés pour obtenir les taux de capture. Nous avons ajusté des modèles linéaires généralisés bayésiens aux densités des différents groupes de taille (81–100, 101–130, 131–160 et >160 mm de CL) de femelles ovigères et non ovigères et de mâles. La capturabilité est fortement affectée par l'année. Des différences de q reliées au sexe et à la taille sont évidentes ches les femelles ovigères, les femelles non ovigères, et les mâles, mais elles sont variables d'une année à l'autre. La taille n'est pas un facteur important de capturabilité des homards dans l'intervalle de 81 et 160 mm de CL. Les homards de Flagg Cove dans l'intervalle de tailles de 81–160 mm ont une capturabilité plus faible que les animaux de taille semblable dans d'autres régions. Nous croyons que cette situation résulte des fortes densités et des tailles plus importantes des homards à Flagg Cove, ce qui entraîne vraisemblablement une augmentation des interactions agonistes et réduit l'entrée des homards dans les casiers.

[Traduit par la Rédaction]

Introduction

The catch rate of animals in traps is a function of animal abundance and catchability. The catchability coefficient relates catch rate directly to abundance (or density) as

$$(1) \qquad q_t = C_t/(f_t D_t)$$

where q is catchability, C is catch, f is effort (number of trap hauls), D is animal density, and t is time. Using this relationship, q can be thought of as the effective fishing area, with units of area per trap (Miller 1975). A related concept is relative catchability (selectivity), which relates the catchability of one component of the population relative to another and does not require absolute estimates of abundance or density. Lobster catchability is a function of size, sex, moult status, behavioral interactions, trap design, and a host of other factors, including temperature and habitat (Miller 1990; Addison and Bannister 1998; Frusher and Hoenig 2001). Understanding the relationship between lobster size and catchability is important given the use of size frequencies

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from commercial traps to estimate indices of mortality and recruitment and the use of trap catch rate as an indicator of abundance. Population trends may be misinterpreted if assessments do not account for changes in catchability, such as those due to a reduction in the component of large lobsters in the population (Frusher et al. 2003).

While there are a number of studies that relate the catchability of *Homarus americanus* to size (Smith 1944; Miller 1995; Tremblay and Smith 2001), these studies have not included the full size range of the American lobster. The American lobster can reach sizes of more than 200 mm carapace length (CL), but harvesting by the trap fisheries in different management areas begins at sizes of 70–84 mm CL in the Canadian Maritimes and off the northeastern United States. With high exploitation rates, large American lobsters (defined here as those greater than 100 mm CL) typically comprise a small fraction of the landed catch, which represents a challenge in studying their catchability. For example, in one of the most productive American lobster fishing areas (southwest Nova Scotia; Lobster Fishing Area (LFA) 34), lobsters greater than 100 mm CL comprise ~11% of the landed catch, with only 5% of the females above 100 mm CL (1999–2000; Pezzack et al. 2001). In the Bay of Fundy (LFAs 35–38), the percentage of American lobsters greater than 100 mm CL in the landed catch is 21% (1999–2000; Lawton et al. 2001*a*), which is among the highest in the Canadian Maritimes.

There is mixed evidence on whether lobster catchability increases or decreases at sizes above 100 mm CL. Miller (1990) suggested that the relationship between the catchability in traps and the size of decapod crustaceans could be described by a logistic curve, with the possible variant of decreased catchability at very large sizes due to mechanical difficulties of large animals actually entering the trap. The southern rock lobster (*Jasus edwardsii*) does not appear to follow this pattern, since catchability increases from 75 to 175 mm CL (Frusher and Hoenig 2001). Previous studies of *H. americanus* indicate catchability increases with size over the range of 50–100 mm CL and then stabilizes or declines (Smith 1944; Miller 1995; Tremblay and Smith 2001). Using data from the offshore American lobster fishery, where sizes tend to be much larger than those in the inshore fishery, Pezzack and Duggan (1995) inferred relative catchability in traps from tag return rates. The plot of return rates versus size was dome-shaped, with tag return rate increasing from 61 to 120 mm CL and then decreasing from 121 to 180 mm CL. These authors noted that their findings were related to gear type, since more large American lobsters were captured in towed trawls than in commercial traps, and also noted that in other trap types (conical top entry), the catch had a higher proportion of American lobsters greater than 130 mm CL. The results of Pezzack and Duggan (1995) support the hypothesis of a decline in *Homarus* catchability above sizes of ~120 mm CL in some common trap types. An alternative explanation for the lower tag returns of large American lobsters observed by Pezzack and Duggan (1995) is that large lobsters are less available to some gear types because of dispersal to lower concentrations.

If direct estimates of densities are possible for a closed lobster population, q can be estimated. In Flagg Cove, off Grand Manan Island in the outer Bay of Fundy (New Brunswick, Canada), an American lobster aggregation with unique population structure (size and sex distribution) is found predictably in late summer (Campbell 1990; Lawton et al. 2001*b*). This aggregation is comprised mainly of large females, and individuals can reside in Flagg Cove for several months, with females hatching and extruding eggs (D. Robichaud and P. Lawton, unpublished data). Here we evaluate trends in catchability over a broad size range and for different sexes or reproductive groups within Flagg Cove: ovigerous females, non-ovigerous females, and males. We extend the methodology developed in Tremblay and Smith (2001) by (*i*) using random effects to account for overdispersion in a Bayesian version of the model for the dive data, (*ii*) linking the dive model directly to a Bayesian version of the model for the trap data, and (*iii*) estimating Bayesian confidence intervals for the catchability estimates. We demonstrate that American lobster catchability in Flagg Cove is lower than that in other locations, perhaps because of the high densities and large sizes in Flagg Cove.

Materials and methods

Flagg Cove, off Grand Manan Island in the outer Bay of Fundy (Fig. 1), was surveyed in September 2001 and 2002. The study area was defined by the dive transects and was approximately 460 m by 325 m. Tide-corrected depths ranged from 1.8 to 13.1 m; at the time of the diving, depths ranged from 4.6 to 18 m. The bottom type was sand–clay with a few old weir poles with kelp attached and a small amount of loose kelp. Cord weed (*Chonda* sp.) was common in the shallower portion of the study area. Six transects were conducted across the study area in each year. Each transect was 300 m in length and was sampled by two divers capturing and measuring the carapace length (CL) of all American lobsters within 1 m of either side of the transect line, for a total area searched of 600 m^2·transect^{-1}. Trapping occurred within 2 days after completing the dive transects. The traps were of commercial design with two compartments and dimensions of 122 cm in length, 60–71 cm in width, and 41–46 cm in height, with two entrance rings of 20 cm in diameter. By regulation, the commercial traps had slots 15 cm long by 4.4 cm high to allow escapement of lobsters below the minimum legal size of 82.5 mm CL. Traps were baited with herring that was fresh or frozen (not recorded) and set for approximately 24 h. Traps were set ~50 m apart in lines across the study site. A total of 23 traps were set each year within the study area.

Data analysis

Tremblay and Smith (2001) used generalized linear models (log-linear models) to estimate density (number per transect) for the dive data as a function of the sex and size of the lobsters and the site at which the survey was conducted. This method has the advantage of evaluating the effect of factors such as size, sex, and site on catchability. The following full factorial model was fit to the data, and terms were included or excluded from the model based on Akaike's information criterion (AIC; Venables and Ripley 2002):

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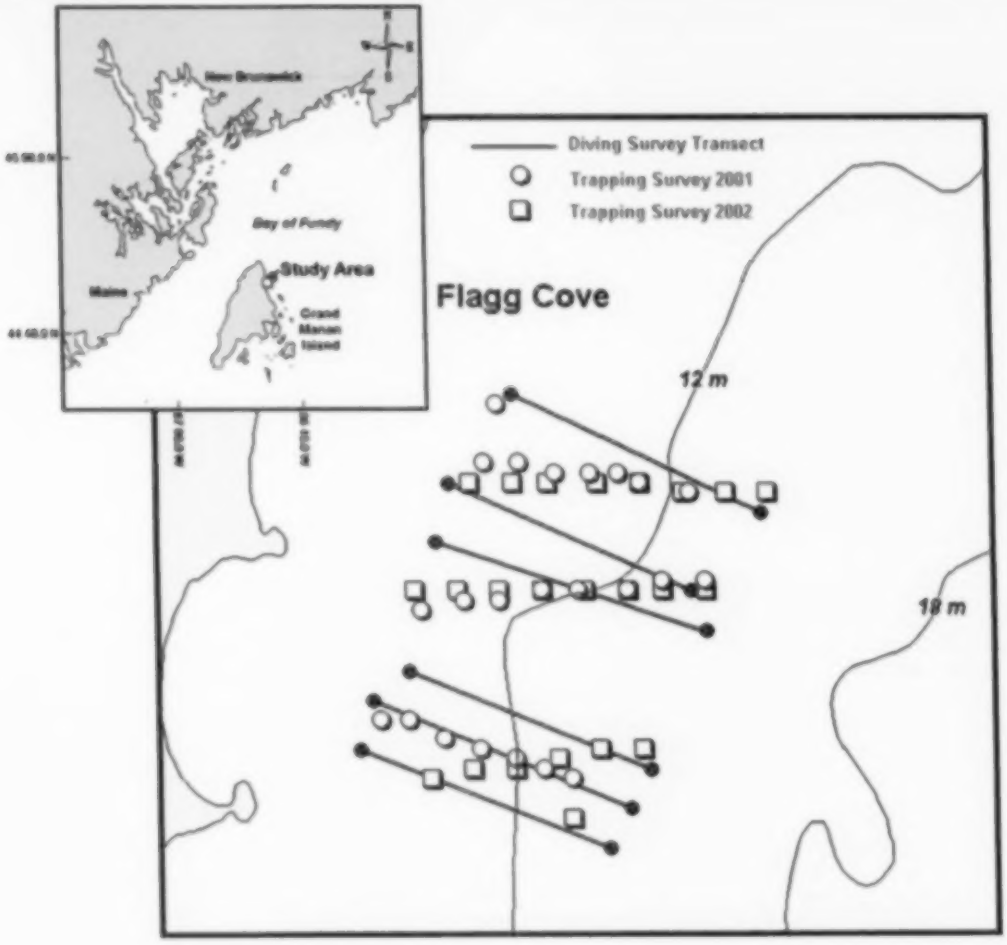
Fig. 1. Map showing dive transects (same in 2001 and 2002), trap positions, and locations of study sites in Flagg Cove, eastern Canada. Chart depths are at lowest normal tide.



$$\text{(2)}\quad \text{no. per transect (or } \hat{D}_t) = \exp(\text{Sex} + \text{Size} + \text{Year} + \text{Sex:Size} + \text{Sex:Year} + \text{Size:Year} + \text{Sex:Size:Year})$$

For the trap data, we are dealing with the following rearrangement of eq. 1:

$$\text{(3)}\quad C_t / f_t = q_t D_t$$

Writing this in a log-linear form similar to eq. 2 gives

$$\text{(4)}\quad \text{no. per trap haul} = \exp(\text{Sex} + \text{Size} + \text{Year} + \text{Sex:Size} + \text{Sex:Year} + \text{Size:Year} + \text{Sex:Size:Year}) \times \hat{D}$$

where $\hat{D}$ is an offset term that corresponds to the predicted number of lobsters per transect from the model in eq. 2. The offset term allows trap catch numbers to be compared for the different terms in the model conditional on their differences from expected catches based on the diving data. For example, if the dive transect data indicated that the 101–130 mm CL size group was twice as numerous as the 81–100 mm CL group, and the trap data showed the same difference, then the inference would be that the two size groups had the same catchability. The offset term is included inside the exponent as $\log(\hat{D})$.

Using the predicted number of lobsters per transect from the dive model for the offset term implies that these predictions are known constants even though they have been predicted from a model with associated error. As such, they underestimate the true variability associated with our estimates of the population available to the trapping experiment. We have introduced a number of modifications to the approach described above to incorporate the error associated with $\hat{D}$.

As in Tremblay and Smith (2001), we began by using a Poisson distribution for fitting the model in eq. 2 to the dive data. In this case, we used a Bayesian approach to generalized linear models (e.g., Dey et al. 2000) with the posterior distribution sampled using Markov chain Monte Carlo

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Table 1. Total counts of different-sized *Homarus americanus* males (M), females without eggs (F), and ovigerous females (OF) in dive transects and trap hauls in each year at the Flagg Cove site.

	Dive transects (n = 6·year^{-1})				Trap hauls (n = 23·year^{-1})			
Carapace length (mm)	M	F	OF	Total	M	F	OF	Total
2001								
<81	4	3	0	7	0	0	0	0
81–100	3	20	10	33	4	6	2	12
101–130	14	67	348	429	4	24	74	102
131–160	7	14	76	97	5	3	16	24
>160	1	1	6	8	1	1	2	4
Total	29	105	440	574	14	34	94	142
2002								
<81	2	6	0	8	1	0	0	1
81–100	3	25	12	40	2	5	2	9
101–130	7	85	276	368	0	2	64	66
131–160	1	5	60	66	2	0	17	19
>160	4	0	8	12	0	0	8	8
Total	17	121	356	494	5	7	91	103

(MCMC) sampling. The results indicated that the data were more variable (or overdispersed) than expected for the Poisson distribution in which the mean is equal to variance. We then used a random effects model to account for the overdispersion by assuming that the Poisson mean (λ) randomly varies over the survey area instead of being a fixed parameter. If we assume that the distribution of the mean follows a gamma distribution with shape parameter θ equal to the scale parameter, then the resultant distribution for the number per transect data is negative binomial with parameters λ and θ (e.g., Venables and Ripley 2002). This assumption can be easily verified for the dive data using a graphical approach by plotting the variances from each Sex, Size, and Year combination against the respective means to test for the following relationship for a negative binomial random variable (see Results):

$$\text{variance} = \lambda + \lambda^2/\theta \quad (5)$$

Terms were included or excluded from the model using the deviance information criterion (DIC; Spiegelhalter et al. 2002), an analogue to AIC.

Trap data were also modelled using a Bayesian form of the Poisson generalized linear model. In this case, the offset term $\hat{D}$ was sampled from the posterior distribution of predicted number of lobsters per transect from the best model for the dive data for each MCMC iteration of the trap data model. The Poisson model was retained here, as there was no evidence for extra-Poisson variation. WinBugs (available at http://www.mrc-bsu.cam.ac.uk/bugs/) was used to fit the Bayesian models. The cut function in WinBugs was used to prevent the MCMC iterations for the trap model from updating the estimates from the dive model. The combination of the two models for the trap data resulted in a mixture model, and as a result, the usual measure of complexity for DIC could not be calculated. In this paper, we use the alternate model complexity estimate of half the posterior variance of the deviance (Gelman et al. 2004).

Following Tremblay and Smith (2001), q is estimated by dividing the predicted number per trap haul by $\hat{D}$, with units of mean number per trap haul divided by mean number per transect. This translates to transects per trap haul, which is converted to units of square metres per trap haul — the same as that used for q in eq. 1 (Tremblay and Smith 2001).

Results

A total of 574 American lobsters were measured in six dive transects in Flagg Cove in 2001, and 494 were measured in 2002 (Table 1). This translates to mean densities of 0.14–0.16 lobster·m^{-2}. Lobsters were not uniformly distributed among or along transects, with portions of some transects having densities of 1 lobster·m^{-2}. Lobsters were typically in bowl-shaped depressions they had excavated or sometimes in the open. In both years, over 94% of the lobsters were females. Most of these females were ovigerous (81% in 2001, 75% in 2002). Lobsters averaged 118 mm CL (2001) and 119 mm CL (2002), with most lobsters between 100 and 130 mm CL (Table 1). The largest lobsters measured in 2001 (189 mm CL) and 2002 (209 mm CL) were ovigerous females.

The total number of American lobster captured in traps was 142 in 2001 and 103 in 2002 (Table 1). Mean CL of lobsters captured in traps was 120 mm CL (2001) and 123 mm CL (2002). The number per trap haul of the different size and sex groups generally reflected the number per transect, with the 101–130 mm CL size group comprising the largest portion of lobsters on the transects as well as those in the traps (Table 1).

The initial fit of the Bayesian Poisson log-linear model to the dive transect data indicated that all of the main effects (Sex, Size, and Year) as well as two-way interactions Sex:Size and Sex:Year were included in the model using DIC. The dispersion parameter, estimated as the weighted sum of the squared Pearson residuals divided by the residual degrees of freedom, should be close to 1.0 for a Poisson distribution, but was much higher at 3.98.

The Bayesian negative binomial log-linear model for the dive data resulted in including main effects for Sex and Size

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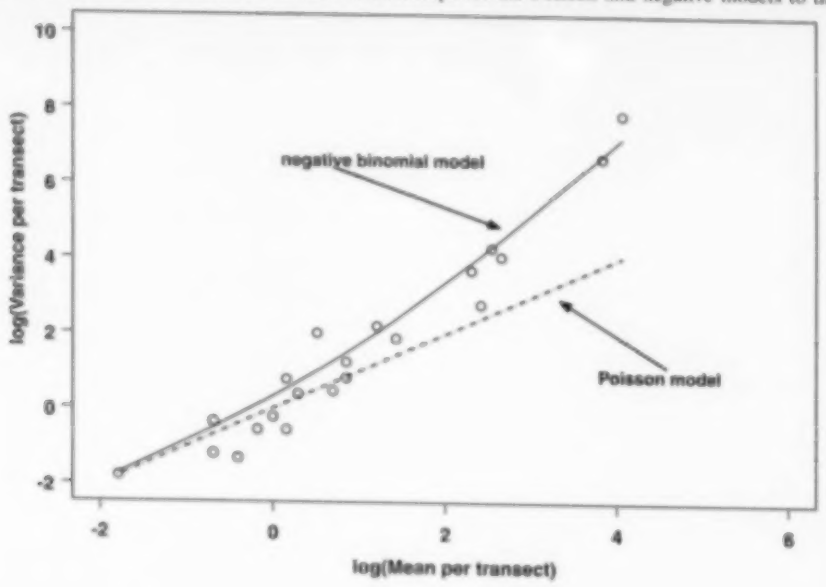
Fig. 2. Comparison of observed and fitted mean-to-variance relationships for the Poisson and negative models to the dive survey data.



only and the two-way interactions term Sex:Size. The posterior mean for θ in eq. 5 was 2.6 (standard error = 0.6). Note that we would expect θ to be quite large (i.e., approach infinity) if a Poisson distribution was more appropriate for these data. A comparison of observed and fitted variances indicated that the negative binomial model was a reasonable fit to these data (Fig. 2). This model was used to predict $\hat{D}$ in eq. 4 for the corresponding levels of Sex, Size, and Sex:Size for the trap data.

Bayesian Poisson log-linear models were fit to the trap data, with the final model chosen by the modified DIC measure as having all three main effects and the two-way interaction terms Size:Year and Sex:Year. The estimate of θ for this model was greater than 10 000, indicating that a Poisson distribution was appropriate for these data.

The predicted catchabilities from the Poisson model are shown (Table 2), ranging from a low of 7 m^2-trap $haul^{-1}$ (non-ovigerous females 101–130 mm CL in 2002) to a high of 111 m^2-trap $haul^{-1}$ for the largest ovigerous females in 2002. Confidence intervals estimated using the posterior distributions were narrow for those groups in high abundance within the study area (both groups of females between 100 and 160 mm CL) and broad for other groups (Fig. 3). The confidence intervals for the catchability estimates show a strong effect due to year, with the effects of sex and size differing in each year (hence the Size:Year and Sex:Year interaction terms in the model). The Year effect was due to elevated catchabilities for two size classes of ovigerous females in 2002 relative to 2001 and due to depressed catchabilities for non-ovigerous females and males in 2002 relative to 2001. In 2001, males had higher and more variable catchabilities than the females, but in 2002 ovigerous females generally had the highest catchabilities. Catchability across sizes within the female and male groups showed no trend in 2001, while in 2002 catchability was lowest for the two middle size classes (81–100 and 131–160 mm CL) and highest for the largest size group.

Table 2. Offset model predictions of catchability q (m^2-trap $haul^{-1}$) for *Homarus americanus* in Flagg Cove.

Carapace length (mm)	Males	Females	Ovigerous females
2001			
81–100	79.0	44.5	34.8
101–130	83.6	47.1	36.2
131–160	90.3	51.7	39.2
>160	66.5	39.0	30.0
2002			
81–100	31.4	16.3	73.4
101–130	13.4	7.0	30.1
131–160	18.2	9.7	41.3
>160	47.0	26.1	111.4

Discussion

Analytical approach

The extension of the methods outlined in Tremblay and Smith (2001) provides several advances. First, the test of different models for the distribution of American lobsters from the dive survey confirms the high aggregation of lobsters in Flagg Cove, expressed here as gamma-distributed random effects. Second, the linking of the dive and trap models incorporates the variability associated with estimates of the expected number of lobsters for the trap survey. Third, the Bayesian confidence intervals for catchability estimates developed here allow the examination of the source of the significant effects due to Sex, Size, and Year.

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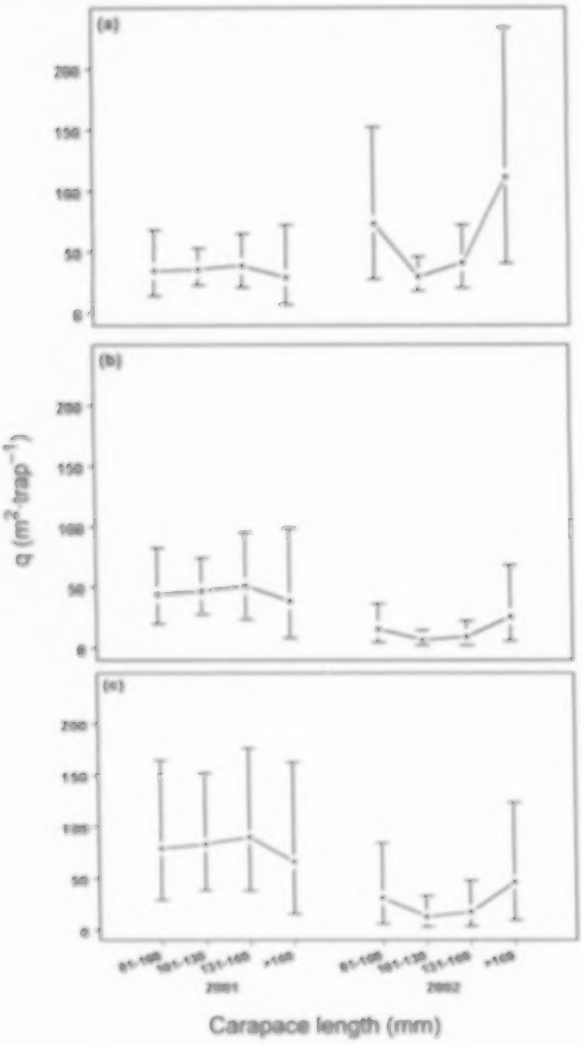
Fig. 3. Catchability (q) estimates for different sexes and sizes of American lobsters (*Homarus americanus*) in Flagg Cove in 2001 and 2002 for (*a*) ovigerous females, (*b*) non-ovigerous females, and (*c*) males. Shown are the posterior means from the Bayesian negative binomial model for the trap data. The upper and lower 95% confidence intervals for Bayesian estimates are based on the posterior distribution.



The finding that the negative binomial model fit the dive data indicated that American lobsters were more aggregated in the Flagg Cove area than in other areas similarly surveyed (Tremblay and Smith 2001) and supports long-term observations of small-scale (tens of metres) differences in the relative spacing of lobsters, particularly ovigerous females (D. Robichaud and P. Lawton, unpublished data). The reason for the greater aggregation of lobsters in Flagg Cove is unclear but lobsters were not aggregated around shelters.

Effects of size, sex and year

The overall mean size of lobsters in traps (120–123 mm CL) was similar to that measured during dive transects (118–119 mm CL). Campbell (1990) reported a similar finding, with the median CL of ovigerous females caught in traps (131 mm) identical to that of lobsters captured while diving. The models developed here show that while the trap catch does deviate from the population structure on the bottom as measured by diving, the effect of size was not strong and the effects of both size and sex were not consistent between years.

This study indicates that American lobster catchability in Flagg Cove is not affected by size over the range of 81–160 mm CL. This size range encompasses all but a very small percentage of American lobsters captured in commercial fisheries. There is an indication of elevated catchability above 160 mm CL in 2002, but the large confidence intervals prevent any conclusions in this regard. The traps and baits used here are a common type used in the commercial fishery, but whether these results apply to other areas, other trap types, and other baits requires further study. Anecdotal information from fishermen indicates that some trap designs and baits are more selective for large lobsters. Evaluation of lobster catchability in the range of trap and bait combinations currently in use in the commercial fishery would be a large and difficult project with the methods used here. Other approaches for measuring relative catchability (e.g., mark–recapture) could be applied, but the calculation of q would require independent estimates of density or abundance.

Ovigerous females were more catchable than non-ovigerous females in 2002, supporting observations by some fishermen that large ovigerous females are aggressive and can kill smaller lobsters in the traps. Although only a low number of males was observed in both diving and trapping, the finding that male catchability was generally higher than non-ovigerous female catchabilty is consistent with previous studies (Miller 1995; Tremblay and Smith 2001). It is not known whether the male–female difference in catchability in Flagg Cove was related to sex difference in molt timing, as appears to be the case elsewhere. With regard to the effect of year on catchability, non-ovigerous females and males were less catchable in 2002 than in 2001 (particularly the 101–130 mm size group), but ovigerous female catchability of two size groups was higher in 2002. The basis for this difference is not clear but may be related to differential foraging activity by the ovigerous females. There were no obvious differences in environmental conditions between the two years that might affect foraging. Temperature and wind were similar in the two years. Tidal height was also similar, since traps were hauled 3 days before spring tides in 2001 and 3–4 days after spring tides in 2002. Investigation of how foraging activity of American lobsters of different sexes and reproductive groups is affected by the spring–neap tidal cycle could help explain the annual differences in catchability observed in the current study.

Low catchability of American lobsters in Flagg Cove

It is of particular interest that the estimates of catchability in the current study were low compared with estimates in other areas. The estimates for Flagg Cove females and males in the 81–100 mm CL size group were below all previous

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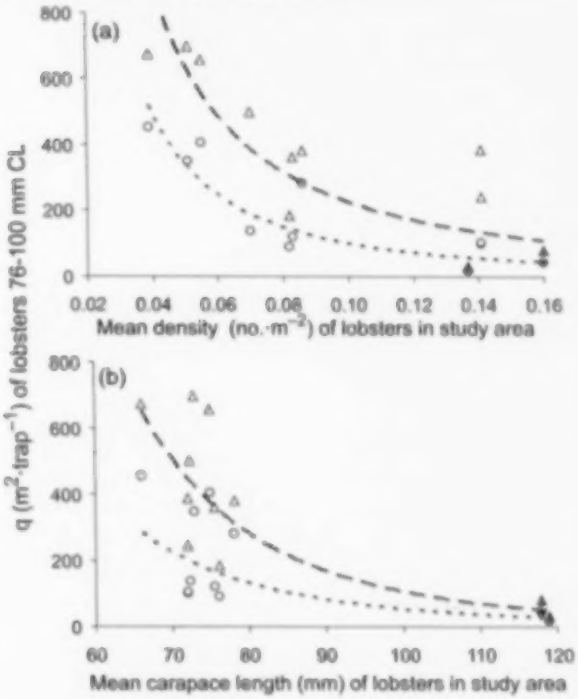
Table 3. Estimates of catchability (q) for *Homarus americanus* and associated trapping data and population characteristics (from diving).

Site		q estimate		Population characteristics		Trapping characteristics		
Area; year(s)	CL (mm)	Male	Female	Mean CL (mm)	Mean no.·m^{-2}	Trap volume (m^3), ring size (cm)	Mean no. trap haul^{-1}	Mean kg·trap haul^{-1}
Flagg Cove; 2001, 2002[a]	81–100	31–79	7–52	118	0.14–0.16	0.30–0.40, 20	3.7,5.9	5.3, 8.0
Lobster Bay (3 sites); 1998[b]	81–100	360–696	120–405	72–75	0.05–0.08	0.17, 13	11.0–16.0	5.1–6.5
Lobster Bay; 1997[c]	81–100	242	101	72	0.14	0.17, 13	13.0	5.1
		386	104			0.30, 15	13.3	
Port L'Hebert; 1987, 1988[d]	80–89	185–380	89–281	76–78	0.08–0.09	0.15, 13	12.0, 15.3	6.0, 8.1
Bay of Chaleur; 1996[e]	76–86	860	453	66	0.04	0.17, 13	9.4	2.8
		747	355			0.17, 17	8.1	2.6
Sydney Harbour; 1987[f]	80–89	500	138	72	0.07	0.15, 13	11.1	5.5

Note: All estimates are for late summer (August–September). Ring size is the diameter of the trap entrance ring. CL, carapace length.

[a]Present study.
[b]Tremblay 2000 (data were regrouped and analyzed using the model-based approach described here).
[c]Tremblay and Smith 2001.
[d]Miller 1995.
[e]Tremblay et al. 1998.

Fig. 4. Catchability estimates for American lobsters (*Homarus americanus*) within the size range of 76–100 mm carapace length (CL) versus (*a*) the density and (*b*) the mean carapace length of lobsters in the study area. Triangles show male data points; circles show female data points. Solid symbols are data points from the current study. Only lobsters >50 mm were included in calculations of means. Source data are provided in Table 3. The fitted lines are power functions.



estimates for American lobsters 70–100 mm CL (Table 3). It is unlikely that the lower catchabilities in the current study were related to differences in trap design, since the large trap volumes and entrance ring sizes used in the current study should have increased the overall catchability of lobsters relative to the other studies cited. The large hoop size of traps used in the current study might have contributed to more escapement of lobsters 81–100 mm CL, but this in itself is an unlikely explanation for the overall lower values of q estimated for Flagg Cove lobsters, since even large lobsters (females 131–160 mm CL) had low catchabilities relative to previous estimates for smaller lobsters.

We hypothesize that the lower catchabilities observed in the current study resulted from the high densities and possibly the large sizes of lobsters at Flagg Cove (Table 3). Both of these factors would tend to increase agonistic interactions around traps. Such interactions are known to have an important effect on lobster catchability (Karnofsky and Price 1989; Jury et al. 2001). Plots of q versus density (Fig. 4*a*) and mean size (Fig. 4*b*) indicate that the catchability of lobsters in the size range of 76–100 mm CL decreases rapidly as a function of these two factors. Unfortunately, in our data set, density is positively correlated with mean size. Since there is higher percentage of variation explained in the plot

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of catchability versus density, and this relationship holds up even without the Flagg Cove data set, we hypothesize that high density is most important as a factor in depressing catchabilities. This hypothesis is supported by experiments on the European lobster, *Homarus gammarus* (Addison and Bannister 1998). In these experiments, traps seeded with lobsters of different sizes had the same inhibitory effect on additional lobsters entering the traps. Several studies have indicated that local size structure in the population affects the relative catchability of American lobsters (Smith 1944; Miller 1995; Frusher and Hoenig 2001), but only Paloheimo (1963) drew a link between lower catchability coefficients and higher lobster density.

An alternative explanation for low catchability of American lobsters in Flagg Cove is that these lobsters are present for reasons other than feeding. Flagg Cove is one of few locations for which there is documentation of such a high proportion of large ovigerous females. Several other coves along the eastern side of Grand Manan Island are known to have similarly skewed sex ratios (from diving surveys similar to those reported in this study), and some deeper-water locations in the upper Bay of Fundy, surveyed by trawl, have also yielded female-skewed sex ratios (P. Lawton and D. Robichaud, unpublished data). These areas presumably provide reproductive advantages. Campbell (1990) suggested that the protected nature of Flagg Cove, coupled with the relatively warm temperatures, provide a good area for egg extrusion and hatching. Others have shown that movement of mature females to warmer, shallower waters in summer accelerates ovary maturation (Ugarte 1994). The apparent reproductive advantages offered by Flagg Cove do not preclude foraging activities. Broken shells of quahogs (*Arctica islandica*) and razor clams (*Ensis* sp.) in Flagg Cove provide signs of lobster feeding (P. Lawton, unpublished data). The very fact that lobsters in Flagg Cove enter traps indicates active foraging. In fact the weight per trap haul in Flagg Cove was at the upper end of observations using similar methods in the same season (Table 3). With the larger mean size and weight of American lobsters in Flagg Cove, fewer lobsters are needed to achieve the same trap catch weight. The traps used in the current study are commonly used in the commercial fishery of the area, and we expect that the catchability trends observed in Flagg Cove would be representative of other local areas with a similar population structure fished at the same time of year.

The results of this study suggest that changes in both density and size structure could affect the reliability of long-term time-series of catch rates. Frusher et al. (2003) suggested this effect for *J. edwardsii*. They showed large southern rock lobsters inhibited smaller lobsters from entering traps and suggested that as large lobsters were removed from the population, smaller lobsters would become more catchable. This would tend to obscure any downward trends in recruitment. If the results of the current study can be generalized, then declines in density or size structure of *H. americanus* stocks may contribute to increased catchability of American lobsters in the size range that currently dominate the commercial catch (81–100 mm CL). Further studies are needed to investigate how sensitive the catchability of *Homarus* is to variation in size structure and population density and how changes in these variables might affect our perception of stock status.

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Density-dependent overwinter survival in young-of-year bluefish (*Pomatomus saltatrix*)? A new approach for assessing stage-structured survival

John Wiedenmann and Timothy E. Essington

Abstract: Identifying the life history stages where density-dependent mortality occurs is essential for understanding fish population dynamics. Here we present an approach to evaluate density-dependent survivorship using catch-at-length data on bluefish (*Pomatomus saltatrix*) from fishery-independent data in the northwest Atlantic, focusing on the stage transition from age-0 to age-1. Abundance indices for each stage were generated by partitioning annual length frequency distributions into catch estimates for individual stages and by using a generalized additive model to standardize survey effort across years. We tested for the existence of density dependence by fitting the data to alternative stage-transition models and using model selection procedures to identify the best-fitting model. We found that that the transition from fall age-0 to age-1 is density dependent and that that significant dampening occurred throughout much of the time series we examined. Estimates of process error were small, suggesting relatively modest variance around the stage-transition model and little interannual variation in survivorship other than that generated by density-dependent effects. Further, we found our conclusions are robust to any potential bias imposed by observation error. We conclude that an improved understanding of bluefish population dynamics might follow from a fuller exploration of the processes dictating overwinter survivorship.

Résumé : Pour comprendre la dynamique de population des poissons, il est essentiel d'identifier les stades du cycle biologique où il se produit de la mortalité liée à la densité. Nous présentons ici une méthodologie pour évaluer la survie liée à la densité à l'aide de données de captures en fonction de la longueur du tassergal (*Pomatomus saltatrix*) provenant d'une banque de données indépendante des pêches commerciales dans le nord-ouest de l'Atlantique et concentrées sur la transition de l'âge 0 à l'âge 1. Nous avons généré des indices d'abondance pour chaque stade ens divisant les distributions annuelles de fréquences de longueurs en estimations de captures pour chacun des stades et en utilisant un modèle additif généralisé afin de standardiser les efforts d'inventaire au cours des années. Nous avons testé l'existence de densité-dépendance en ajustant les données à des modèles de rechange de transition de stades et en utilisant des méthodes de sélection de modèles afin de trouver le modèle le mieux ajusté. Nous trouvons que la transition de l'âge 0 à l'automne à l'âge 1 est liée à la densité et qu'il se produit un amortissement significatif sur presque toute la série chronologique examinée. Les estimations des erreurs de processus sont petites, ce qui indique qu'il y a une variance relativement faible dans le modèle de transition des stades et peu de variation dans la survie autre que celle générée par les effets liés à la densité. De plus, nos conclusions sont robustes vis-à-vis toute déformation potentielle imposée par les erreurs d'observation. En conclusion, une exploration plus complète des processus responsables de la survie à l'hiver pourrait aboutir à une meilleure compréhension de la dynamique de population des tassergals.

[Traduit par la Rédaction]

Introduction

Nearly all fish populations undergo distinct and pronounced shifts in habitats and trophic position throughout ontogeny, and it is widely recognized that some of these life history stages are more important than others in their influences on the overall population dynamics of a species. Demographic modelers have produced a well-developed approach to identify life history stages that have the greatest influence on population growth rates (Caswell 2001) through linear models that describe the survivorship and transition of individuals through life history stages. Alternatively, fisheries ecologists have focused on those life history stages that have the greatest effect on the variation in cohort strength and population dynamics. This latter perspective, initiated by the pioneering observations of Hjort (1914), has produced a large body of evidence suggesting that cohort strength is dictated by survivorship through critical life history periods. These life history periods commonly take place early in fish life histories (Myers and Cadigan 1993*a*; Leggett and DeBlois 1994; Beaugrand et al. 2003), but later life history stages may also be important (Peterman et al. 1988; Pace et al. 1993). Identifying

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these crucial life history stages is key for achieving an improved understanding of controls on fish population dynamics.

Recruitment variability is dictated by variance in survivorship through multiple life history stages. Two kinds of variance are important: those dictated by stochastic, environmental effects and those dictated by endogenous, density-dependent effects. Thus, anomalous survivorship through an early life history stage may not directly translate into anomalous recruitment because of stochastic processes operating during later stages or because of density-dependent effects that act to dampen variation (Myers and Cadigan 1993*b*; Kimmerer et al. 2000; Fromentin et al. 2001). Consequently, the search for density-dependent survivorship and identification of where the life history stages occur and what ecological processes mediate them has been a focus of fisheries ecology for several years.

Identifying density-dependent survivorship in particular, and critical life stages in general, has been hampered by a paucity of adequate data to test hypotheses. Long time series of detailed data are needed to assess the relationship between stage-specific abundance indices. Further, even well-designed survey data may not control for all extraneous environmental forces that dictate catchability (Walters and Martell 2004), introducing observation error into these indices. It is well known that this error can introduce biased estimates of density dependence (Walters and Ludwig 1981) and obscure the ability to recognize true variation in cohort dynamics. Finally, it is often difficult to identify and partition individuals into each life history stage based on the type of data (e.g., length–frequency) commonly collected.

In this paper, we develop an analytical approach to address these problems in assessing cohort dynamics and survivorship through life history stages. We develop this approach using data on bluefish, *Pomatomus saltatrix*, collected by the Northeast Fisheries Science Center (NEFSC, Woods Hole, Massachusetts) as part of their annual bottom trawl survey. Bluefish have well-defined life history stages, with two cohorts (spring- and summer-spawned) produced each year (Kendall and Walford 1979; Collins and Stender 1987). It is generally believed that these cohorts are spawned in two distinct events by a single stock (Chiarella and Conover 1990; Graves et al. 1992; Austin et al. 1999). Both cohorts of juveniles occupy the same nursery areas until fall, at which point they move out over the continental shelf and begin a southward migration to the South Atlantic Bight. Age-1 fish return to the Mid-Atlantic Bight in the spring, occupying both coastal and pelagic waters (Fahay et al. 1999). The NEFSC collects primarily juvenile (both spring- and summer-spawned) and age-1 bluefish as they move out over the continental shelf and begin migrating to the South Atlantic Bight (see Salerno et al. (2001) for the spatial distribution of bluefish by age collected in the NEFSC survey). Although density-dependent survivorship may happen at any time during bluefish ontogeny, the available data lend themselves to analysis of overwinter survivorship through considering the transition from fall juvenile to age-1 stages.

Materials and methods

Data

The NEFSC bottom trawl survey has been conducted annually in spring and autumn since 1962, sampling bottom waters of the continental shelf primarily from Cape Hatteras, North Carolina, to the Gulf of Maine. We used data from the fall survey only because the timing of the survey coincided with the southern migration of bluefish over the continental shelf. In 1972, the survey was expanded to include inshore stations with depths <27 m. Because Munch and Conover (2000) noted that the majority of young-of-the-year (YOY) bluefish were caught in depths <27 m, we only used data collected from 1972 to 2001. The survey used a stratified random sampling design, so the same sites were not sampled each year.

These data have three components. For each tow, spatial (latitude and longitude), temporal (time of day and date), and environmental (bottom temperature and sampling depth) data were collected. The data also indicate the total number of bluefish captured in each tow. Finally, lengths were measured for nearly all bluefish that were captured. From 1985 to 1996, bluefish ages were estimated from the scales of 3341 fish collected in this survey (Salerno et al. 2001). Scale sampling was based on length, but samples were not representative of the lengths found in each tow. As a result, these data could not be used to estimate the relative proportions of age-0 and age-1 bluefish in a given year's catch. However, this age-at-length data provided useful guidelines for length at age-1 in the methods described below.

Approach to analyzing data

Our approach required three separate steps. First, we generated annual abundance indices for each stage based on total catch, length–frequency distributions, and the sampling efficiency of each year. Second, we used these abundance indices to fit alternative stage-transition models to determine the magnitude of density-dependent survivorship. Lastly, we evaluated whether the magnitude of density dependence could be attributed to observation error in our catch estimates.

Generating annual abundance indices

Generating abundance indices involved two separate procedures. First, we partitioned the total catch in each year t into catches of each stage ($C_k(t)$), where $k = 1, 2, 3$ for summer-spawned age-0, spring-spawned age-0, and age-1, respectively, based on analysis of length–frequency distributions. The second step was to account for interannual variation in sampling efficiency of the survey imposed by variation in the time, location, and environmental characteristics of the trawl stations.

For each year, we pooled all of the length data and constructed annual length–frequency distributions. To partition catches into each life history stage, we assumed that each annual length–frequency distribution consisted of the sum of three separate length–frequency distributions (each representing the sizes and abundance for individual stages). The task was to therefore estimate parameters of these distributions for each stage and then estimate the total numbers of individuals captured for each stage.

We developed a model to partition composite length–frequency distributions into stages that assumes that the catch of fish with length l ($C(t,l)$) is the sum of the catch of each group times the proportion of each group that has a length equal to l ($p(t,l)$):

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$$C(t,l) = \sum_{k=1}^{3} C_k(t) p_k(t,l)$$

(1)

$$p_k(t,l) = \int_{l-1}^{l} \exp(v_k(t))\, dl$$

where $v_k(t)$ is ~$N(\ln[\mu_k(t)], \sigma_k^2(t))$.

We assumed that each life history stage in each year has a length–frequency distribution that follows a log-normal distribution, with a mean $\mu_k(t)$ and standard deviation $\sigma_k(t)$. We assumed that $\mu_1 < \mu_2 < \mu_3$ (i.e., summer-spawned age-0 fish were smallest, followed by spring-spawned age-0 fish, and then age-1 fish) (McBride and Conover 1991; Fahay et al. 1999; Conover et al. 2003). This model, therefore, has nine parameters for each year ($C_k(t)$, $\mu_k(t)$, $\sigma_k^2(t)$; $k = 1,2,3$), with $C_k(t)$ being the parameters of primary interest. For each annual length–frequency distribution, we found the maximum likelihood estimates of $\mu_k(t)$, $\sigma_k(t)$, and $C_k(t)$ numerically using Matlab (The MathWorks, Inc., Natick, Massachusetts). Estimates of $\mu_3(t)$ (mean size at age-1) were constrained to be consistent with the limited NEFSC age–length data available. These data indicated that age-1 sizes ranged between 17 and 45 cm, but the majority of samples ranged between 25 and 40 cm. Based on these observations, we constrained $\mu_3(t)$ to be at least 25 cm. We then used likelihood profiles to calculate 95% confidence intervals for C_k (Hilborn and Mangel 1997). Observed and predicted length distributions for all years examined are reported by Wiedenmann (2004).

The catch estimates calculated above do not provide a direct measure of abundance because the sampling locations and timing varied each year in the survey. We therefore needed to calculate the effective sampling effort for each year based on the characteristics of the tow locations. Bluefish catchability was calculated by correlating bluefish catch rate with tow conditions (depth, temperature, date, location). The relationships between catch rate and tow conditions are likely nonlinear (Bigelow et al. 1999), so we used a generalized additive model (GAM) to determine this relationship. GAMs are analogous to regression analysis, except that the shape of the relationship is not constrained to any parametric function (Hastie and Tibshirani 1990; Swartzman et al. 1992). The GAM predicts catch rate as a function of each tow variable from a locally weighted least-squares regression (Hastie and Tibshirani 1990). In fisheries, GAMs have been used for a variety of purposes, including smoothing of abundance time series (Bigelow et al. 1999), estimating recruitment success based on stock structure (Cardinale and Arrhenius 2000), and determining the spatial and environmental effects on catch rate in fisheries-independent surveys (Swartzman et al. 1992, 1995).

For this analysis, we pooled all of the NEFSC survey data, log-transformed the catch data (ln(catch + 1)), and calculated the residual catch rate for each tow as ln(catch + 1) – $\bar{Y}$, where $\bar{Y}$ is the mean of the log-transformed catch rates. We omitted catches of bluefish with lengths >45 cm, because this exceeds the typical size of age-1 or younger fish. The tow variables we examined were time of day, date, depth, bottom temperature, latitude, and longitude. All predictor variables were rounded or converted for computational ease. Latitude and longitude were rounded to the nearest quarter of a degree. Temperature was rounded to the nearest half a degree Celsius. Time of day was rounded to the nearest half hour (in decimal units) and then converted to sin ($t\pi$) to make time continuous across days. Tows made at depths >35 m, as well as those missing one or more predictor variables, were omitted. In total, 3157 tows were examined in the GAM.

Statistically significant predictors were determined in a forward stepwise selection procedure, with significance determined using Akaike's information criteria (AIC; Burnham and Anderson 1998). Predictors were sequentially incorporated into the model if they could improve the model fit, while being penalized for increasing the degrees of freedom of the model.

The GAM results were then used to weight the effort of each tow (q_i) at collecting bluefish. Because the GAM predicts ln(catch + 1) – $\bar{Y}$, the estimate of effort for the ith tow in non-log-transformed units is

$$q_i = \exp\left(\sum_j S(x_j) + \bar{Y}\right) - 1 \qquad (2)$$

where $\sum_j S(x_j)$ represents the predicted catch rate based on the values of the j predictors (depth, temp, etc.). When tows were missing one or more predictor values, we used the average smoothed value for the missing predictor for that year. The effective effort of sampling in each year ($E(t)$) was equal to $\sum q_i(t)$, and annual catch per unit effort (CPUE) for each stage ($N_k(t)$) was calculated as the catch divided by the effective efforts ($N_k(t) = C_k(t)/E(t)$). Tows with a negative q_i were omitted from the calculation of $E(t)$.

Fitting stage-transition models

The above methods produced estimates of relative abundance for the summer juvenile (N_1), spring juvenile (N_2), and age-1 (N_3) stages. We quantified the degree of density dependence in the age-0 to age-1 transition by fitting the data to stage-transition models. A stage-transition model describes the relationship between the abundance of some early life history stage and the resulting abundance of some defined later life history stage. Thus, the standard stock–recruit models commonly employed in fisheries analyses are special cases of these stage-transition models, wherein the dependent variable is some measure of reproductive output (e.g., recruits, egg production, etc.). We therefore used equivalent mathematical forms for these stage-transition models.

The stage-transition models were fit in two ways. The first combined all juvenile (e.g., spring- and summer-spawned) abundance indices and related those to age-1 abundance the following year. The contribution of summer-spawned juveniles to age-1 recruitment is known be variable and was shown to be small in most of the years we examined where summer-spawned juveniles were abundant (Conover et al. 2003). As a result, we considered a second model that related only spring-spawned juveniles to age-1 abundance. The general model used was

$$N_3(t+1) = f(N_x(t)) \qquad (3)$$

where $N_x(t)$ is either total juvenile abundance ($N_1 + N_2$) or spring-spawned juvenile abundance ($N_2(t)$), and $f(N_x(t))$ is one of three stage-transition models:

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(4) Linear: $f(N_s(t)) = \alpha N_s(t)$

Ricker: $f(N_s(t)) = \alpha(N_s(t))\exp(-\beta N_s(t))$

Beverton–Holt: $f(N_s(t)) = \alpha N_s(t)/(1 + \beta N_s(t))$

These functions can be fit to data in two ways: (*i*) the best estimates of $N_s(t)$ and $N_s(t + 1)$ are treated as fixed quantities known without error; (*ii*) observation error in both dependent and independent variables are explicitly incorporated into the analyses. Here we use both methods for the sake of comparison and to show that our conclusions are robust to the type of method chosen.

When $N_s(t)$ and $N_s(t + 1)$ are considered to be fixed quantities, the likelihood function for each pair of $N_s(t)$ and $N_s(t + 1)$, denoted i, is

$$(5) \qquad L(i) = \frac{1}{\sqrt{2\pi\sigma^2}} \exp\left\{-\frac{[f(N_s(i)) - N_s(i)]^2}{2\sigma^2}\right\}$$

where σ^2 represents the total error variance. Because this model does not include terms for observation error, we refer to this as the reduced likelihood function.

We can account for the observation error resulting from the partitioning of catches by explicitly considering it in the likelihood function. Each residual, $f(N_s(i)) - \hat{N}_s(i)$ (denoted $R(i)$), results from the sum of some observation error, $u(i)$, and some process error, $v(i)$. The $u(i)$ and $v(i)$ are not directly observable, but we assume that $v(i)$ are identically and independently distributed with unknown variance σ_v^2, and that each $u(i)$ is drawn from a random distribution with variance $\sigma_s^2(i)$. We have estimates of observation error variance for each data pair i ($\sigma_s^2(i)$ and $\sigma_{\hat{s}}^2(i)$) from the confidence interval calculation above, assuming that each confidence interval is four standard deviations wide. This assumption presumes that the uncertainty in abundance estimates can be described by a normal distribution, which was a reasonable approximation to the observed likelihood profiles. The full likelihood function that includes these observation errors is

$$(6) \qquad L(i) = \frac{(2\pi\sigma_{\hat{s}}^2(i)\sigma_v^2)^{-1}}{\sqrt{2\pi\sigma_s^2(i)}} \int\int \exp\left(-\frac{(N_s(i)\hat{N}_s(i))^2}{2\sigma_s^2(i)} - \frac{(R(i) - v)^2}{2\sigma_{\hat{s}}^2(i)} - \frac{v^2}{2\sigma_v^2}\right) dv\, dN_s$$

For both likelihood functions used here, the best-fitting model was determined using AIC with a correction for a small sample size (AIC_c) (Johnson and Omland 2004). Maximum likelihood parameter estimates of stage-transition function were determined numerically.

Because observation error can produce biased estimates of β when data are considered to be known without error (Walters and Ludwig 1981), we ran a Monte Carlo simulation to determine the amount of observation error that would be needed to produce an estimate of the Beverton–Holt density-dependent parameter β equal to or greater than the observed estimate if the true value was zero. This procedure generated random data sets based on specified process and observation error from a linear stage-transition relationship. The simulation used each artificially generated data set and applied the standard likelihood and weighted likelihood function described above to estimate β in the Beverton–Holt model. From this simulation, the expected bias in the estimate of β was calculated based on estimates of process and observation error, as well as the proportion of β estimates that were greater than or equal to the observed estimate.

Table 1. Predicted estimates of mean size (μ_k) and standard deviation (σ_k) for the summer juvenile ($k = 1$), spring juvenile ($k = 2$), and age-1 ($k = 3$) stages.

Year	μ_1	σ_1	μ_2	σ_2	μ_3	σ_3
1972	14.0	0.11	21.0	0.09	29.0	0.04
1973	10.0	0.12	20.5	0.08	27.5	0.12
1974	4.5	0.11	20.0	0.09	31.0	0.14
1975	13.0	0.11	19.0	0.06	28.0	0.10
1976	4.5	0.17	18.5	0.09	29.5	0.12
1977	5.5	0.15	19.0	0.10	27.5	0.22
1978	8.5	0.15	17.0	0.10	29.0	0.10
1979	14.0	0.18	20.0	0.08	29.0	0.12
1980	9.5	0.07	21.5	0.07	28.5	0.20
1981	12.5	0.07	19.5	0.10	27.5	0.08
1982	5.5	0.18	20.5	0.09	30.5	0.10
1983	13.0	0.30	20.0	0.11	34.0	0.12
1984	9.0	0.01	19.0	0.19	—	—
1985	14.0	0.15	18.0	0.15	32.5	0.14
1986	11.5	0.10	16.5	0.10	27.5	0.15
1987	9.0	0.09	18.5	0.07	29.0	0.12
1988	10.5	0.19	16.0	0.16	26.0	0.12
1989	4.5	0.06	19.0	0.14	31.0	0.12
1990	13.0	0.10	20.5	0.12	31.5	0.08
1991	11.5	0.01	18.5	0.07	31.5	0.10
1992	7.5	0.14	21.0	0.09	29.5	0.08
1993	12.0	0.20	21.5	0.16	39.0	0.10
1994	14.0	0.15	22.5	0.08	29.0	0.06
1995	8.5	0.22	20.5	0.08	29.5	0.13
1996	8.5	0.17	17.0	0.11	30.0	0.10
1997	9.5	0.13	16.0	0.04	30.0	0.06
1998	13.0	0.12	21.0	0.10	29.0	0.10
1999	15.0	0.13	23.0	0.10	31.0	0.10
2000	12.5	0.18	18.5	0.15	29.5	0.08
2001	12.0	0.12	17.5	0.07	30.5	0.12

Results

Stage-structured catch

All estimates of μ_k and σ_k are found in Table 1. Mean size estimates for the summer cohort showed considerably more interannual variation than estimates for the spring cohort and age-1 bluefish (CV = 30.7% for the summer cohort compared with 9.6% for the spring cohort and 8.0% for the age-1 fish). For the summer cohort, estimates ranged from 4.5 to 15 cm, and spring cohort estimates ranged from 16 to 23 cm. These estimates are consistent with the autumn lengths reported by McBride and Conover (1991; 3–13 cm for the summer cohort, 13–27 cm for the spring cohort). Mean size estimates for age 1 were between 26 and 39 cm, with all but one estimate falling between 27 and 34 cm. For 1984, the model estimated that the mean size for age-1 bluefish was 21 cm, well below our lower constraint of 25 cm. Therefore, we

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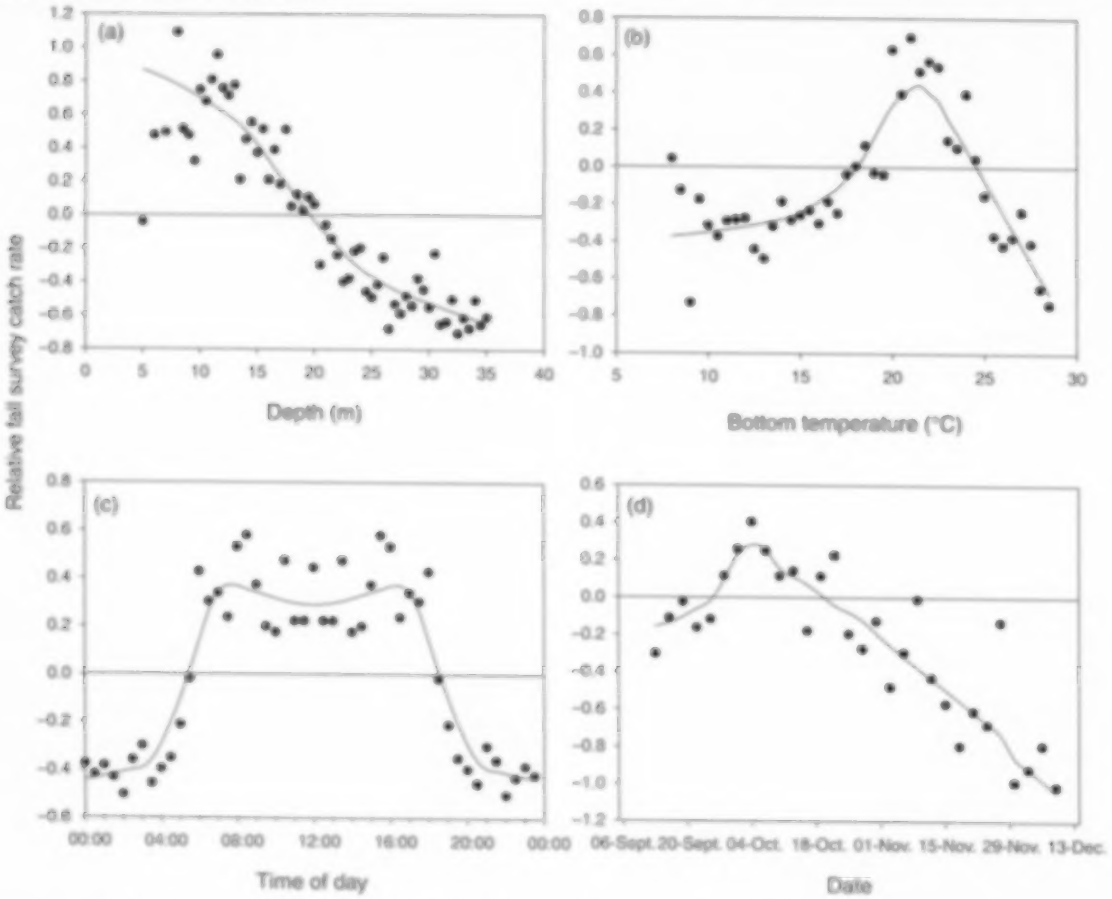
Fig. 1. Relative catch rate from the fall Northeast Fisheries Science Center survey vs. (*a*) sampling depth, (*b*) bottom temperature, (*c*) time of day, and (*d*) sampling date. The solid line represents the generalized additive model smoothed fit, and the points are the mean values at each observation. The line at 0 represents mean catch, with positive values indicating above-average catches and negative values indicating below-average catches.



considered the catch estimates from this year to be unreliable and removed them from all subsequent analyses.

Effective effort

GAM results indicated that juvenile and age-1 bluefish catch rates were best predicted by depth, with above-average catch rates occurring in shallow (<20 m) water (Fig. 1*a*). Temperature was the next best predictor. Above-average catches occurred between 18.5 and 24.5 °C, with peak catches at 21.5 °C (Fig. 1*b*). Time of day was the third best predictor, with above-average catch rates during daylight (0530 to 1830; Fig. 1*c*). Trawl date was the final environmental variable that could significantly improve the prediction of catch rates, with above-average rates between 21 September and 12 October (Fig. 1*d*).

Of the 11 953 tows conducted in the fall from 1972 to 2001, 6371 had a positive estimate of effective effort (i.e., were conducted at times and places where bluefish might be caught). Annual estimates of effective effort fluctuated widely throughout the time period, ranging 135–522. Uncorrected estimates of effort (number of tows per year) displayed different trends with respect to effective effort, such that uncorrected and corrected CPUE were only weakly correlated (r^2 = 0.12; Fig. 2). The differences between raw and corrected effort estimates were largely due to interannual differences in depths sampled. When we reestimated uncorrected effort by removing all tows conducted at depths >35 m (where the predicted catchability was close to zero), the two CPUE time series were more closely correlated (r^2 = 0.56).

Density dependence

The analysis of stage-transition patterns between late-fall juvenile and age-1 suggest density-dependent control on age-1 recruitment (Figs. 3*a* and 3*b*). For the analysis that considered all juveniles (spring- and summer-spawned) contributing to age-1 recruitment, both likelihood methods indicated that the Beverton–Holt and Ricker models provided a significantly better fit over the linear model (Table 2). When we used the reduced likelihood function, the Ricker and Beverton–Holt models provided nearly equivalent fits, while the Ricker model indicated a slightly better fit from the Beverton–Holt model. For both the Ricker and Beverton–

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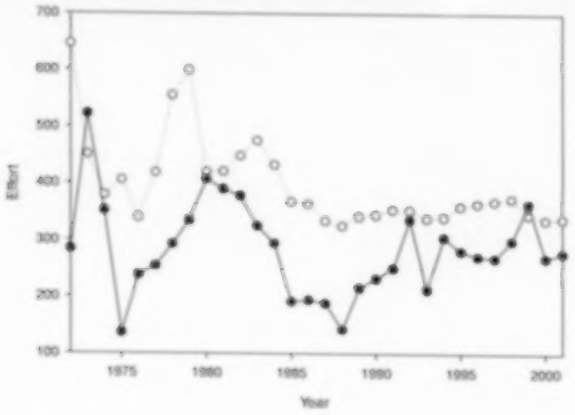
Fig. 2. Annual estimates of generalized additive model-generated effective effort (solid circle, solid line) and uncorrected effort (number of tows per year; open circles, dotted line).



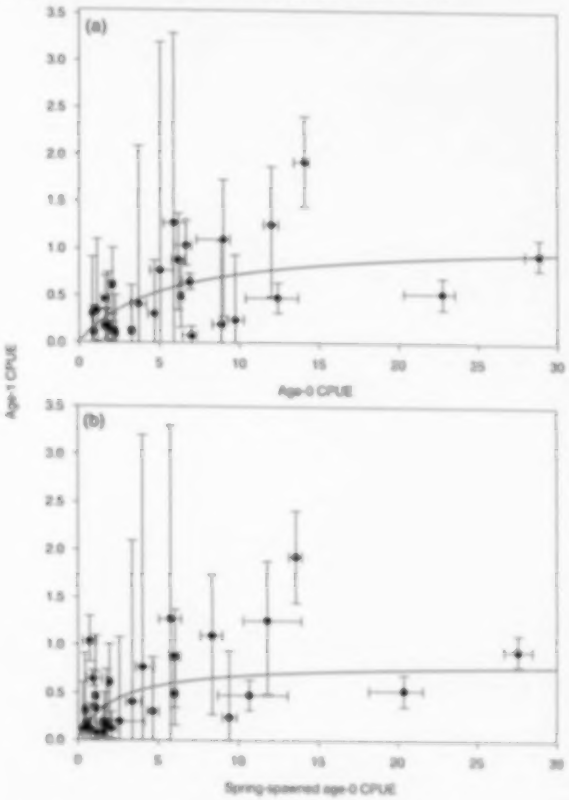
Holt models fit using the full likelihood function, estimates of the density-dependent parameter were reduced (for the Beverton–Holt, β declined from 0.187 to 0.153; for the Ricker, β declined from 0.057 to 0.047).

The stage-transition models that only considered the spring-spawned cohort suggested even stronger density-dependent control on age-1 recruitment (Fig. 3*b*). For both likelihood functions, the Beverton–Holt model provided the best fit to the data (Table 2), but the Ricker model also provided a significantly better fit over liner model. The magnitude of the density-dependent parameters were also much greater than those estimated from the total YOY vs. age-1 stage-transition models, with the Beverton–Holt β term nearly twice as large in the spring-spawned vs. age-1 transition model. Also, models fit with the full likelihood function also indicated that the two density-dependent models provided a significantly better fit to the data than the linear model.

Because the AIC for the Beverton–Holt model was generally lowest, we used this model for all further analyses. The maximum likelihood parameter estimates generated from the reduced likelihood function fit to the total YOY vs. age-1 data indicated density dependence that maintained age-1 recruitment within a factor of two (from 0.58 to 0.95; number per effective tow) over the upper 50th percentile of juvenile abundances. However, these estimates predicted variable recruitment (from 0.15 to 0.58 (number per effective tow)) over the lower 50th percentile of juvenile abundance (Fig. 3*a*). The parameter estimates derived by only considering the spring-spawned cohort indicated density dependence that maintained recruitment from 0.43 to 0.97 age-1/effective effort over the upper 50th percentile of spring-spawned YOY abundances and from 0.1 to 0.43 over the lower 50th percentile of YOY abundances.

Because Conover et al. (2003) noted that the 1994 summer-spawned cohort contributed significantly to age-1 recruitment, we also estimated the Beverton–Holt β parameter by adjusting the recruitment to represent the spring-spawned contribution to age-1 recruitment in this year and by also excluding this year altogether. The adjusted 1994 data point (N_3 = 0.62) yielded β = 0.46; while excluding this year altogether, β = 0.315. Thus, the higher values of β observed when fitting the spring-spawned to age-1 stage transition is not likely due to the higher contribution of summer-spawned juveniles during this year.

Fig. 3. (*a*) Total age-0 catch per unit effort (CPUE; spring + summer) vs. age-1 CPUE the following year and (*b*) spring juvenile CPUE only vs. age-1 CPUE the following year. Error bars represent 95% confidence intervals for each estimate. The solid line is the relationship predicted from the reduced maximum likelihood estimates from the Beverton–Holt model.



The estimated process error variances derived from the full likelihood model were small. Under the best fitting Beverton–Holt models, process error variance was 0.012 and 0.019 for the total YOY and spring-spawned YOY stage-transition models, respectively. In contrast, the mean age-1 observation error variance was 0.088. This suggests relatively modest variance around the stage-transition model and little interannual variation in overwinter mortality other than that generated by density-dependent effects.

Because the reduced likelihood function does not consider observation error and the full likelihood function only considers the observation error that is attributable to partitioning cohorts on the basis of size, we conducted a Monte Carlo simulation to determine the magnitude of observation error needed to cause an estimate of the Beverton–Holt β equal to or larger than our observed estimate when the true stage-transition relationship was linear. This Monte Carlo simulation indicated that the mean observation error variance (in both age-1 and YOY abundance) would have to exceed 0.3 to cause an estimate of β equal to or greater than our

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Table 2. Parameter estimates of stage-transition models relating (*i*) total young-of-the-year abundance (spring + summer juvenile) and (*ii*) spring juvenile abundance to age-1 abundance for both the reduced and full likelihood functions.

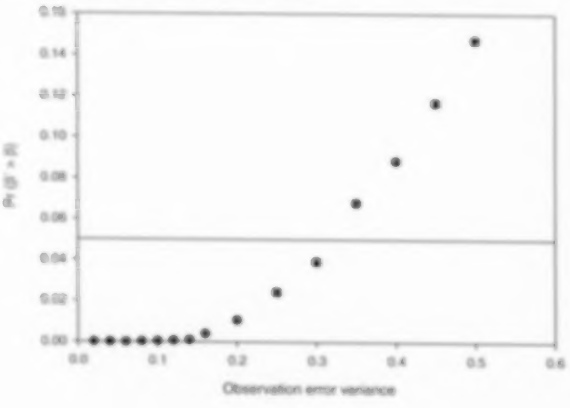
		Model		
Function	Parameter*	Linear	Beverton–Holt	Ricker
Spring + summer juvenile abundance				
Reduced likelihood	α	0.054	0.21	0.143
	β	—	0.187	0.057
	NLL	16.86	11.34	11.19
	AIC	35.88	27.18	26.88
Full likelihood	α	0.066	0.1517	0.1029
	β	—	0.1527	0.0474
	σ_ε^2	0.06	0.012	0.012
	NLL	−63.18	−87.47	−86.63
	AIC	−124.2	−170.44	−168.76
Spring juvenile abundance				
Reduced likelihood	α	0.184	0.342	0.19
	β	—	0.316	0.0698
	NLL	18.24	11.62	11.8
	AIC	38.64	27.74	28.1
Full likelihood	α	0.0477	0.384	0.157
	β	—	0.478	0.066
	σ_ε^2	0.051	0.019	0.018
	NLL	−67.14	−82.05	−77.83
	AIC	−132.12	−159.6	−151.16

*α and β are parameters found in eq. 4; σ_ε^2, process error variance found in eq. 6; NLL, negative log-likelihood; AIC, Akaike's information criteria.

observed value more than 5% of the time (Fig. 4). In comparison, the mean observation error estimated from partitioning cohorts on the basis of size was 0.089, 0.122, and 0.087 for total YOY, spring-spawned YOY, and age-1 abundance indices, respectively. Thus, there would have to be additional sources of observation error that increased the variance by a factor of 3 to induce a 5% chance of getting an erroneously large estimate of β when the true value was 0.

Finally, if competition were a major source of density-dependent mortality in bluefish, then we might expect to see a lower mean size at age-1 when juvenile abundance was particularly high in the previous year. We evaluated this expectation by comparing body sizes following years of high and low juvenile abundances. High abundance years were defined as those that would, on average, produce age-1 recruitment equal to one-half of the asymptotic recruitment as predicted by the Beverton–Holt model (total juvenile CPUE = 5.26; n = 14). Although the mean age-1 length was lower following years of high juvenile abundance (mean length = 29.6) compared with years following low juvenile abundance (mean length = 30.6), these differences were not statistically significant (t = −1.06, df = 26, p = 0.15).

Fig. 4. The probability of observing a β estimate from the Monte Carlo simulation greater than our observed estimate ($\hat{\beta} > \beta$) for different levels of observation error.



Discussion

We used a novel approach to partition length–frequency data into each life history stage, coupled that with a method to account for interannual variation in sampling efficiency, and found evidence supporting the hypothesis that over-winter survivorship of bluefish is dictated in part by density-dependent processes. Furthermore, this conclusion was not likely biased by observation error in our estimates of juvenile and age-1 abundance; the amount of bias that we would expect based on our estimates of observation error was small compared with the magnitude of the density-dependence parameter. Consequently, we found that the stage-transition curve relating spring-spawned juveniles to age-1 recruits predicted that age-1 recruitment reached one-half of the maximum value (e.g., the half-saturation point) at a juvenile CPUE of 2.12. Notably, 14 of the 28 years showed juvenile abundances above this value, indicating considerable damp-

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ening of potentially large variation in early life history survivorship over one-half of the time series.

Ever since the development of the critical period hypothesis by Hjort (1914), fish ecologists have focused on the early life stages as sources of recruitment variability. Many studies have concluded that variation in the adult population likely arises from variation in larval mortality. Evidence supporting this conclusion derives from syntheses of published reports (Leggett and DeBlois 1994), quantitative meta-analysis of several populations (Myers and Cadigan 1993*a*), as well as mechanistic studies that link shifts in food quality with changes in stock productivity (Beaugrand et al. 2003). Our study adds to a growing list of works that suggest that later life history stages may also have important controls on fish population dynamics. Peterman et al. (1988) found no relationship between early life history stages and recruitment of northern anchovy (*Engraulis mordax*) and concluded that variability in some later stage was sufficient to destroy the relationship. Pace et al. (1993) also found that recruitment to the juvenile stage was unrelated to larval abundance for white perch (*Morone americana*) and striped bass (*Morone saxatilis*) in the Hudson River estuary, and Kimmerer et al. (2000) reached similar conclusions for striped bass recruitment in San Francisco Bay.

We initially started this study as a broader exploration of the controls on bluefish population dynamics and examined larval data collected in the Marine Resources, Monitoring and Prediction (MARMAP) survey. However, data from this survey were only collected from 1977 to 1987, and the larvae were collected primarily in the summer months. Unfortunately, these were years in which the summer cohort was essentially absent from the population and also a period when the adult population remained relatively stable, preventing any inferences on larval controls on the population. Nonetheless, the existence of density dependence in the juvenile stage indicates reduced larval control on the population dynamics of bluefish.

We identify at least two important implications of our findings for the future study and management of bluefish in the northwest Atlantic. The first is that fall juvenile CPUE measured in survey data may not provide a precise measure of cohort strength, particularly when abundances are high. Notably, these data are presently used to "tune" bluefish stock assessments (Lazar and Gibson 2002). The sensitivity of this approach to density-dependent overwinter survivorship seems warranted. The second issue is that first year overwinter survival could be a key period dictating bluefish recruitment success. Although competition may be one potential source of this density-dependent mortality, we found no difference in the mean size at age-1 following years of low and high juvenile abundance. Supporting this result, Salerno et al. (2001) found no difference in growth rates of bluefish of all ages between 1985 and 1986, a period that contained both times of low and high abundance. Additional information regarding the sources of mortality on these stages remains scarce, thus highlighting our incomplete understanding of bluefish ecology. Our best understanding of bluefish is restricted to the estuarine juvenile stage in the Mid-Atlantic Bight, with little known about the overwintering period. Therefore, an improved understanding of bluefish population dynamics might best be achieved by complimenting ongoing work with research focused on the overwintering period.

Munch and Conover (2000) used the same data set to examine questions regarding bluefish recruitment. The present study extends their analyses by developing a quantitative method for partitioning the catch, standardizing catch rates based on trawl characteristics, and fitting stock–recruit models to explicitly test for density dependence. We highlight some of the methodological advances made in the present study and their potential applications to other data sets below.

The mode-selection algorithm used to partition catches into life history stages based on length allowed us to quantify stage catches without setting arbitrary cutoffs and provided us with estimates of observation uncertainty. Splitting composite length distributions into individual stage or age classes is not new, however, as Bhattacharya (1967) showed how composite distributions can be divided into a series of normal distributions. Our approach refined this method by adopting a likelihood approach that permitted estimation of observation error variance. Getting these estimates of observation error was essential, because it allowed us to address any bias in our estimates of density dependence associated with this partitioning algorithm. We found this approach to be highly effective in providing objective estimates of cohort sizes, and we suspect this will be similarly useful for other species that have stages with broad, overlapping length–frequency distributions.

One possible source of bias in our mode-partitioning method might exist if bluefish exhibit a bimodal length distribution at age-1. If the mean size of summer-spawned bluefish at age-1 is similar to that of the spring-spawned juveniles (at the time of collection in the NEFSC survey), then our mode-separation technique would overestimate spring-spawned juvenile abundance and underestimate age-1 abundance in years when the summer cohort contributed to the age-1 pool. However, this result is unlikely to effect our conclusion of density-dependent overwinter survivorship. Summer-spawned fish were only collected in great abundance in the NEFSC survey from 1990 onward. Conover et al. (2003) examined the relative contribution of the summer-spawned cohort to the adult stock by back-calculating length at age-1 from fish collected between 1992 and 1999. They found that most adults collected had back-calculated lengths at age-1 corresponding to lengths of spring-spawned bluefish, suggesting that the summer cohort contributed little to the adult stock during this period.

Yearly variation in survey timing and station location can introduce considerable variation in catchability, even when following a statistically rigorous survey design such as that used in the NEFSC survey. GAMs provide a useful way to determine which tow variables affect catch rates, which enables us to partition out the variance in catches at a fine scale by explicitly accounting for the variation in catch rates caused by differences in the location, timing, temperature, and depth of individual samples. This robust approach allows for the reduction of error caused by inconsistency in fishery-independent surveys. For bluefish, the difference in our GAM-corrected and uncorrected (tows per year) abundance indices was striking, with little correlation between the two measures. When we removed

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tows at depths that the GAM predicted were not effective at catching bluefish (>35 m) from the uncorrected effort, there was a much improved (but not full) correlation between corrected and uncorrected CPUE. The GAM, therefore, provided objectively derived and simple criteria for estimating annual effort from these survey data. The attributes of bluefish that make their catchability particularly variable is their specificity to particular depth strata and their seasonal migration patterns. Abundance estimates of species that are more widely distributed across habitats may not be as sensitive to these measures. GAMs are also useful for examining the behavioral ecology of a species such as bluefish. Although most of what is shown in Fig. 1 is well known (i.e., bluefish in general prefer warm water, juvenile and age-1 fish migrate out of estuaries in early fall, and stay close to the coast as they migrate south), little has been documented on the vertical distribution of young bluefish. The results shown in Fig. 1*d* suggest that juvenile and age-1 bluefish move off the bottom at night, at least during their fall migration to the South Atlantic Bight.

Some have questioned the validity of using a bottom trawl survey to estimate bluefish abundance because of the potentially poor catchability of these fishes with the trawl gear (Munch and Conover 2000; Salerno et al. 2001). However, the effectiveness of the gear will only bias our results if there was variation in catchability based on size and if this relationship changed over time. Unfortunately, we know of no way to test for these potential sources of bias from this data set. We do note that the survey used the same gear and vessels for the duration of the time series used in the present study.

In summary, we conclude that the stage transition from the late juvenile to age-1 stage is density dependent and may serve to dampen annual variation in early life history survivorship, particularly when juveniles are very abundant. Because of the potential importance of this life stage, an improved understanding of bluefish population dynamics might best be achieved by complimenting ongoing research of nearshore juvenile ecology with studies on overwintering fishes.

It is clear that attempts to understand fluctuations in marine fish populations would benefit from a broader focus on all life history stages, as processes acting on later stages may reduce the contributions of earlier stages to the adult population. We presented one possible method to examine stage-structured survival, but a variety of methods may be used, so long as they account for the many sources of observation error that may bias results.

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Reporting and shedding rate estimates from tag-recovery experiments on Atlantic cod (*Gadus morhua*) in coastal Newfoundland

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Abstract: It is important to have good estimates of tag reporting rates when inferring exploitation rates and other mortality rates from tagging experiments. We estimate the reporting rates of single- and double-tagged Atlantic cod (*Gadus morhua*) caught in commercial fisheries around coastal Newfoundland, Canada, based on an extensive series of multi-reward tagging experiments conducted during 1997–2004. Reporting rates for single-tagged cod varied from 58% to almost 100%, with significant temporal and spatial variability. The odds of reporting a double-tagged cod was almost double that of a single-tagged cod. Returns from double-tagged cod allow us to estimate tag shedding rates. Tag shedding rates suggested that 22% of fish lost their tag during their first year at liberty; subsequently, tag shedding rates were much lower (<10%). We also found that twice as many fish lost tags when the tags were attached anteriorly at the base of the first dorsal fin compared with a position more towards the posterior end.

Résumé : Lorsque l'on déduit les taux d'exploitation ou d'autres taux de mortalité à l'aide d'expériences de marquage, il est important d'obtenir de bonnes estimations des taux de signalisation des étiquettes. Nous estimons les taux de signalisation de morues franches (*Gadus morhua*) marquées d'une ou de deux étiquettes dans les pêches commerciales autour de la côte de Terre-Neuve, Canada, à partir d'une grande série d'expériences de marquage assorties de diverses primes menées en 1997–2004. Les taux de signalisation des morues porteuses d'une seule étiquette varient de 58 % à presque 100 %, avec une variabilité temporelle et spatiale significative. La probabilité qu'une morue marquée de deux étiquettes soit signalée est presque le double de celle d'une morue avec une seule étiquette. Les retours obtenus à partir des morues porteuses de deux étiquettes nous ont permis d'estimer les taux de perte d'étiquettes. Les taux de perte indiquent que 22 % des poissons perdent leur étiquette au cours de leur première année en liberté; par la suite, les taux de perte des étiquettes sont beaucoup plus bas (<10 %). De plus, deux fois plus de poissons perdent leur étiquette lorsque celle-ci est fixée en avant à la base de la première nageoire dorsale que lorsqu'elle est placée plus vers la région postérieure du corps.

[Traduit par la Rédaction]

Introduction

Tagging experiments are commonly used to estimate important wildlife population parameters such as exploitation (i.e., harvest), survival, and migrations rates. There are numerous literature references related to tagging, and many of these are reviewed by Schwarz and Seber (1999). Tag-return experiments are the most practical and feasible method to assess some fish stocks that are difficult to survey using synoptic trawl or acoustic methods. For example, Atlantic cod (*Gadus morhua*, hereafter referred to as cod) along the northeast coast of Newfoundland overwinter in deep, fiordic-like bays and then disperse along the coast during the summer and autumn to feed. The abundance of these fish is difficult to measure using trawl surveys because a large part of the stock range in the inshore is not trawlable. Synoptic acoustic techniques are also difficult to use because of the large areas involved and because the fish behavior during much of the year makes them difficult to detect acoustically. A comprehensive tagging program in these inshore areas began in 1997. Estimates of exploitation rates have been produced from these tagging studies and have been valuable in recent stock assessments (Fisheries and Oceans Canada 2002). These estimates are independent of the reported commercial catch. However, there are practical problems when determining exploitation rates from tag returns. In this paper, we consider the important problems of estimating tag reporting rates and, secondarily, tag shedding rates.

Reporting rates can be estimated by various methods; for example, multiple releases per year, seeding experiments, fishery surveys, scientific observer reports, and the use of high-reward tags. Pollock et al. (2001) and Pollock et al. (2002*a*) reviewed the latter method, but also included many references and a discussion of advantages and disadvantages for some of the other methods. Pollock et al. (2002*b*), Hearn

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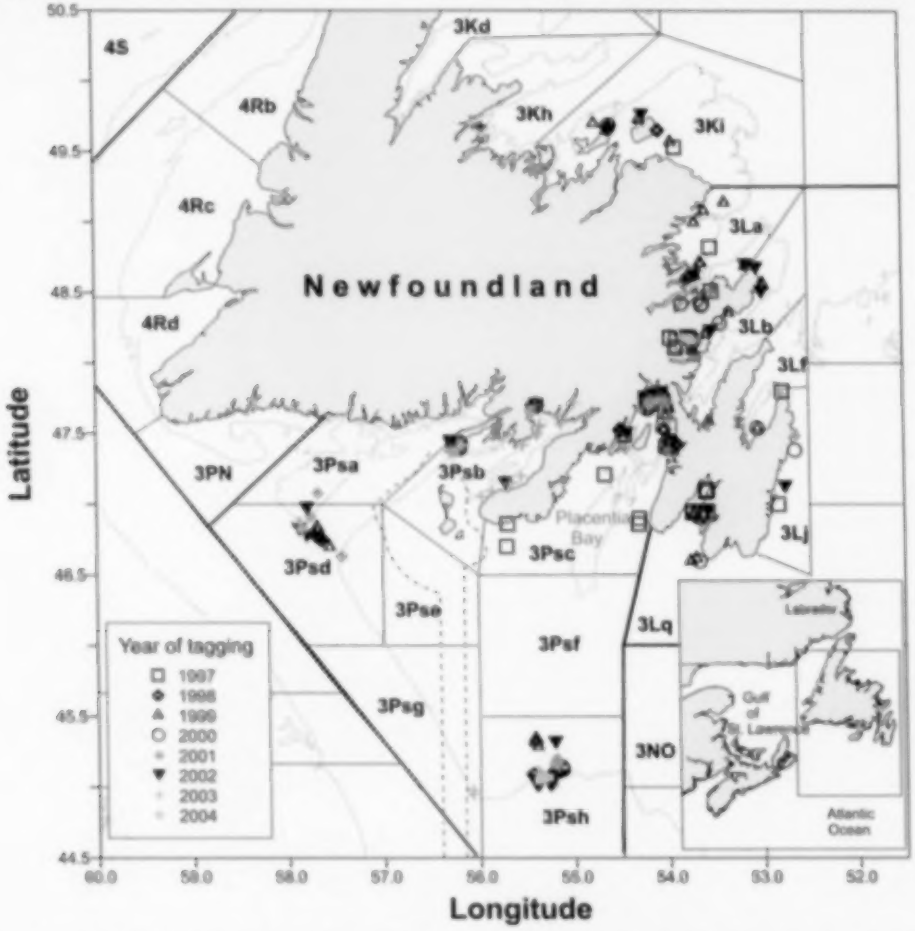
Fig. 1. Locations where Atlantic cod (*Gadus morhua*) were tagged and released around Newfoundland during 1997–2004. Solid lines are boundaries of Northwest Atlantic Fisheries Organization (NAFO) unit areas; the broken line is the boundary of the French economic zone; the light grey line is the 200 m depth contour. Inset shows study location off the east coast of Canada.



et al. (1998), and Hearn et al. (2003) considered other methods to estimate reporting rates.

In our tagging experiments, we utilized high-reward tags to estimate reporting rates. We released batches of fish with low- and high-reward tags, with the value of the high reward sufficient to ensure a reporting rate of 1 or very near 1. This enabled us to estimate and compute confidence intervals for the low-reward tag reporting rates. We also assess spatial and temporal variation in the reporting rates, and where necessary we estimate reporting rates separately. Spatial and temporal variation in reporting rates have been observed in waterfowl banding studies (e.g., Nichols et al. 1995) and in some marine fisheries tagging studies (e.g., Hampton 1997). Some fish were double-tagged in our experiments to provide information about tag loss rates; however, we also expect that double tags result in different reporting rates than single tags because the reward for returning two tags is double the single tag reward. We consider efficient estimation of the double tag reporting rates. Cadigan and Brattey (2004) presented methods to estimate tag loss rates and reporting rates for our experiments, although primarily their focus was on estimating tag loss rates. In this paper, we develop a simplified statistical model and improved estimates of reporting rates compared with Cadigan and Brattey (2004). In addition, we update the tag retention estimates presented in Cadigan and Brattey (2004) based on additional tag returns.

Material and methods

Tagging experiments

Tagging experiments were conducted on cod in coastal waters off insular Newfoundland, Canada, during 1997–2004. The experiments were conducted in Northwest Atlantic Fisheries Organization (NAFO) Subdivision 3Ps and Divisions 3KL (Fig. 1). Most cod for tagging were captured with handlines equipped with feathered hooks, but some trap-caught and otter-trawled cod were also tagged. Live cod caught with handlines or otter trawls were initially placed directly into a 900 L tank equipped with running seawater. Trap-caught fish were held at the surface, dipped from the trap catch, and tagged and released immediately. The length of each cod (nearest cm) was recorded. Only cod >45 cm in

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fork length and in excellent condition were used. Experienced technicians tagged most of the cod, and individuals undergoing training were carefully supervised. Fish were tagged with one or two 6.3 cm t-bar anchor tags (Floy Tag Co., Seattle, Washington) inserted at the base of the first dorsal fin. Double tags were spaced ~3 cm apart on the same side of the fish; one tag was inserted in the same location as single-tagged fish, which is referred to as position 1. The location of the second posterior tag is referred to as position 2.

A reward scheme was used to encourage those participating in the fishery to return cod tags and recapture information. The reward for returning a standard tag, and any tags from previous studies, was CAN$10. Cod were tagged with one standard yellow ($10 reward), two standard yellow ($20 reward for returning both tags), or one high-reward pink tag ($100 reward). During initial experiments, tags were applied in the sequence one pink tag, nine single yellow tags, one pink tag, nine double yellow tags. This insured that fish with different tag types were thoroughly mixed. The proportion of high-reward and double tags was reduced starting in 1999. However, the tags were always applied in an alternating sequence to achieve good mixing. Tags had the value of the reward printed on them, as well as a unique serial number and a return address.

The reward scheme was advertised widely by means of posters illustrating the size, color, position of attachment of tags, and reward values; these were sent to all fish plants processing cod in Newfoundland. Plant managers were contacted to confirm that posters were received and prominently displayed and that plant workers processing cod were familiar with the details of the tagging program. Local fisheries officers distributed posters widely among fishing communities. Pre-addressed cod tag return envelopes were sent to processing plants, local fisheries officers, observers, port samplers, and commercial groundfish license holders. The reward scheme was also advertised on local radio prior to and during the commercial and recreational fisheries. Fishermen's logbooks also contained a detailed description of the tagging program. All individuals who returned tags were sent a standard letter describing the date, size, and location where the fish was tagged along with a request to confirm recapture information as well as provide any further recapture details.

We refer to the release of a batch of tagged fish over a 1- to 3-week period at a specific site as an experiment (x), and the release time as t_x. We analyzed tag returns from 119 experiments conducted during 1997–2003. These were experiments that in addition to using single, low-reward tags also used at least some double or high-reward tags. If only single low- or high-reward tags were used in an experiment and for a length class, then subsequent recaptures did not provide information about tag loss or reporting rates (see High reward method). Length is important because the fisheries are size-selective. We pooled lengths into 3 cm classes to increase the number of tags that could be used to estimate reporting rates, and because we did not feel that selectivity varied much within 3 cm length classes. Note that only two tagging experiments were conducted late in 2004, and no recaptures were reported in this year, so we do not consider the 2004 experiments further. The number of fish tagged in the experiments during 1997–2003 ranged from 5 to 3828, with an average of 721. Twenty-seven technicians performed the tagging; however, most of the tagging (73%) was performed by two technicians. The tagged fish caught ranged in length from 41 to 125 cm, although only 10% of recaptures were smaller than 47 cm, and 10% were larger than 86 cm. Three tag types were used: single (j = S), double (j = DD), and high reward (j = H). Two additional tag types can be observed at capture: a single tag from position 1 of a double release (j = D1) and a single tag from position 2 (j = D2).

The annual numbers of fish released and returned that we use to estimate reporting rates are presented (Table 1). A total of 16 193 tags were returned from 84 648 releases. The average time-at-liberty was 68 weeks, and the 10th and 90th percentiles were 9 and 141 weeks, respectively. Starting in 2001, almost no fish were double-tagged because we concluded that sufficient numbers of double-tagged fish were released to adequately assess tag loss rates. Approximately 11% of fish were tagged with high-reward tags in all years. Note that substantially more double-tagged fish were caught with one tag in position 2 compared with position 1. The total numbers returned for positions 1 and 2 were 256 and 532, respectively.

High reward method

Virtually all of the information about reporting rates can be obtained from a statistical likelihood for the observed tag-return types, conditional on the total number of recaptures of all types of tags. This approach is convenient because many parameters related to harvest and migration rates can be eliminated in the conditional likelihood, and this allows us to estimate reporting rates and tag shedding rates separately from these other parameters. The specific conditional distribution that is best to use depends on the sources of variability or factors that affect tag returns. The basic approach is to pool recaptures within levels of the factors and then use the conditional distribution of the pooled tag returns; hence, the appropriate amount of pooling depends on the factors that affect tag recoveries. We describe this in more detail in the Discussion section. The first step is to construct a statistical model to describe tag recaptures in terms of numbers released, exploitations rates, etc. Then we use this model to define the appropriate conditional distribution to use for inferences (i.e., estimates and confidence intervals) about reporting rates. The factors we include in our model are (1) tag-type j, (2) tagging experiment x, (3) release length l, (4) recapture region h, and (5) recapture time t. The rationale for including these factors in our model is described below.

Reporting rates depend on factors 1, 4, and 5, but not on factors 2 and 3. We assume fish length (l) does not affect reporting rates, and experiment (x) does not either because fisherman have no knowledge of when and where a fish was released. Let λ_{jht} be the reporting rate for the jth tag type. We assume that the reporting rate for high-reward tags (type H) is 1. Arguments for this important assumption are presented in the Discussion. We assume that the reporting rates for types S, D1, and D2 tags are equal and denoted as λ_{sht} (s = single). This assumption is reasonable because fishermen cannot distinguish these three tag types. The reporting rate for type DD tags, denoted as λ_{dht} (d = double), is assumed to be greater than λ_{sht} because the reward for returning two tags is double the reward for returning one tag. We test the statistical significance of the difference between

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Table 1. Annual number of Atlantic cod (*Gadus morhua*) released (bold rows) and recaptured that were used to estimate reporting and tag loss rates.

	Tag type					
Year	S	D1	D2	DD	H	Total
1997	**4 753**	**—**	**—**	**3 012**	**923**	**8 688**
1997	160	7	10	139	47	363
1998	225	22	25	139	64	475
1999	259	31	52	186	80	608
2000	142	12	22	82	31	289
2001	52	7	5	28	4	96
2002	21	2	5	10	5	43
2003	12	1	3	4	1	21
2004	2	0	1	1	0	4
1998	**4 065**	**—**	**—**	**3 993**	**1 971**	**10 029**
1998	186	14	28	189	145	562
1999	346	47	109	347	269	1 118
2000	196	18	65	177	96	552
2001	62	9	18	58	42	189
2002	22	3	9	23	10	67
2003	12	2	5	6	3	28
2004	6	0	2	1	0	9
1999	**12 063**	**—**	**—**	**1 799**	**1 673**	**15 535**
1999	1 111	8	18	122	165	1 424
2000	860	21	30	104	128	1 143
2001	403	3	26	46	43	521
2002	168	5	7	27	21	228
2003	69	5	0	10	12	96
2004	20	0	0	1	5	26
2000	**9 305**	**—**	**—**	**1 961**	**334**	**11 600**
2000	647	10	22	151	18	848
2001	676	12	36	113	9	846
2002	297	11	16	38	7	369
2003	117	2	13	19	1	152
2004	38	2	1	2	0	43
2001	**11 979**	**—**	**—**	**1**	**1 356**	**13 336**
2001	1 188	0	0	0	98	1 286
2002	799	0	0	0	81	880
2003	303	0	0	0	26	329
2004	74	0	0	0	5	79
2002	**12 777**	**—**	**—**	**2**	**1 702**	**14 481**
2002	888	0	1	0	108	997
2003	820	0	0	0	104	924
2004	282	0	0	0	26	308
2003	**9 339**	**—**	**—**	**2**	**1 638**	**10 979**
2003	527	0	0	0	134	661
2004	483	0	0	0	126	609

Note: S, single tags; DD, double tags; H, high-reward tags; D1, one tag in position 1; D2, one tag in position 2.

single and double tag reporting rates in the next section. Reporting rates also tend to be region- (h) and time- (t) specific for a variety of reasons; for example, there may be temporal and regional differences in fishermen's awareness of the tagging program and their willingness to return low reward tags. However, we do not estimate different λs for every time interval and region. A smaller number are estimated for region and time (i.e., calender year) categories that we select based on statistical tests. We feel it is reasonable to assume that reporting rates are constant within a year. The specific region and year categories that we use are described later in this section.

Let R_{jxlht} be a random variable for the number of type j tag returns from length l fish tagged in experiment x and recaptured in region h at time t. We model the expected number of tag returns as $E(R_{jxlht}) = \lambda_{jht}\mu_{lht}N_{jxlht}$, where μ_{lht} is the fishery exploitation rate and N_{jxlht} is the number of tagged fish available to the fishery. Exploitation rates depend on length because the fishing gears used are size-selective. Exploitation rates vary spatially (i.e., h) and temporally (i.e., t) because of different quotas, gear types, and other management regulations. We assume that the exploitation of tagged fish does not depend on the type of tag, which is reasonable for populations like cod. We model the number of single-tagged fish available to the fishery (i.e., j = S or H) at time t in region h as $N_{jxlht} = \Phi_{x1}\xi_{xlht}N_{jxl}$, where Φ_{x1} is the fraction of the tagged population that have not lost their tag during the time-at-liberty $t - t_x$, N_{jxl} is the number of length l fish with tag type j that were released in experiment x, and ξ_{xlht} is the fraction of the releases that have survived and are present in fishing region h. The fraction ξ_{xlht} is a complicated and high-dimensional parameter that depends on x through $t - t_x$. It involves the cumulative, length-dependent exploitation from t_x to t that a fish experiences as it grows to its capture size and as it migrates through different regions.

If tags on double-tagged fish are lost independently of each other, then the expected number of type DD-tagged fish available to the fishery is $N_{DDxlht} = \Phi_{x1}\Phi_{x2}\xi_{xlht}N_{DDxl}$, where Φ_{x2} is the retention probability for the tag at position 2. The expected numbers of D1 and D2 fish are $N_{D1xlht} = \Phi_{x1}(1 - \Phi_{x2})\xi_{xlht}N_{DDxl}$ and $N_{D2xlht} = \Phi_{x2}(1 - \Phi_{x1})\xi_{xlht}N_{DDxl}$, respectively. It is common to assume that $\Phi_1 = \Phi_2$ for all t; however, Cadigan and Brattey (2004) showed that this was not true for the tagged cod we considered, and this was why twice as many type D2 tags were returned compared with type D1 tags. We use the same models for Φ_1 and Φ_2 as described in Cadigan and Brattey (2004) and in the Tag loss rates section of the Results, and these involve four parameters to estimate.

We use the maximum likelihood method for estimation based on the conditional distribution of R_{jxlht} given $R_{\cdot xlht} = \sum_j R_{jxlht}$, which we assume is multinomial and involves only Φs and λs. The multinomial maximum likelihood estimates (MLEs) can also be obtained by minimizing the multinomial deviance function (McCullagh and Nelder 1989), which is

$$D(\lambda, \Phi) = 2\sum_{jxlht} r_{jxlht}\log|r_{jxlht}/E_v(R_{jxlht})|$$

where $E_v(R_{jxlht}) = E(R_{jxlht}|R_{\cdot xlht} = r_{\cdot xlht}) = r_{\cdot xlht}p_{jxlht}$ and $p_{jxlht} = \Pr(R_{jxlht}|R_{\cdot xlht})$. The difference in the deviance between nested models can be directly compared with a χ^2 distribution when assessing if improvement in fit is statistically significant ($P < 0.05$). The tag type probabilities are

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$$(1)\qquad p_{jxlhr} \propto \begin{cases} \lambda_{xlhr}\Phi_{x1}N_{Sxl} & j = \mathrm{S} \\ \lambda_{xlhr}\Phi_{x1}(1-\Phi_{x2})N_{\mathrm{DD}xl} & j = \mathrm{D1} \\ \lambda_{xlhr}\Phi_{x2}(1-\Phi_{x1})N_{\mathrm{DD}xl} & j = \mathrm{D2} \\ \lambda_{xlhr}\Phi_{x2}\Phi_{x1}N_{\mathrm{DD}xl} & j = \mathrm{DD} \\ \Phi_{x1}N_{\mathrm{H}xl} & j = \mathrm{H} \end{cases}$$

The proportionality $\propto$ is such that $\Sigma_j p_{jxlhr} = 1$ for each x, l, h, and t.

The tag type probabilities involve two basic modelling components: the reporting rate model and the tag loss rate model. Reporting rates are modelled as a combination of three factors for tag type (s or d), region (h), or time (t). The basic reporting rate model we use is $\log[\lambda/(1-\lambda)] = \mathrm{logit}(\lambda)$ = tag type + region + time + interactions, where the terms on the right indicate the type of parameters. This logit transformation insures that $0 < \lambda < 1$. To simplify the model, we use fairly broad region and time categories (described below) and we only use statistically significant interaction terms. The tag type parameter for single tags is set to a reference level of zero. The double tag type parameter is described in more detail at the end of this section. We use deviance tests and profile deviance confidence intervals to test for significant interactions. The tag loss model involves tag type and time-at-liberty ($t - t_x$), which is treated as a continuous covariate. Note that Φ_{xt} depends on x only via $t - t_x$. Tag loss rates are not modelled separately for different experiments x. This model is described further in the Results section. The parameters for Φ_{xt} are estimated simultaneously with reporting rate parameters.

We present some SAS/OR®, SAS/STAT, and BASE SAS computer code to estimate reporting rates for a simple example (Appendix A). The data for the example are also included. This is essentially the same code that we use to estimate reporting rates for the cod data.

The unit areas we use (Fig. 1) are the finest spatial scale at which we could assess variability in reporting rates. This is because many tags that are returned do not have more detailed information about the capture location. However, we had insufficient returns to estimate annual reporting rates in each unit area, so we combined some areas and estimated reporting rates for eight regions (described in Table 2). Virtually all of our tag returns have come from these regions, although the 3NO region is currently under a cod fishing moratorium, and only 11 returns have come from there. We expect that reporting rates are more similar within than between these regions in part because there is likely greater communication among fishermen within these regions. There were too few returns in most regions during 1997 to estimate regional reporting rates, so we estimated a combined reporting rate for 1997–1998. Also, because of fishing moratoria, there were few returns in 3K_IN, 3L_INS, and 3PN_4RS in 2003–2004, so we estimated combined reporting rates in these regions and years. The region with the most tag returns was Placentia Bay (3Ps_PB; Table 3). In some other regions, the number of returns was fairly small (e.g., 3Ps_BB, 3PN_4RS). One of the reasons we test for the statistical significance of between-cell differences in reporting rates is to pool cells whose differences are not statistically significant (i.e., large P values), which should increase the reliability of the estimates.

Table 2. Reporting rate regions.

Region	NAFO unit area
3K_IN	3Kd, 3Kh, 3Ki
3L_INN	3La, 3Lb
3L_INS	3Lf, 3Lj, 3Lq
3Ps_PB	3Psc
3Ps_FB	3Psb
3Ps_BB	3Psa, 3Psd
OFF_SH	3Pse–3Psh, all of 3NO
3PN_4RS	3Pn, 4Ra–4Rd, 4Sv–4Sw

Note: NAFO, Northwest Atlantic Fisheries Organization.

The number of cod recaptured with two tags (i.e., type DD) is usually much fewer than the number of cod caught with one tag. This means that less information is available to estimate the reporting rates of double tags (λ_d) compared with single tags (λ_s), and this results in less reliable estimates of λ_d than λ_s. For example, in Cadigan and Brattey (2004), some of the λ_d estimates seemed too high relative to the λ_s estimates, although the standard errors were large enough to suggest that more reasonable values were also consistent with their data. Better estimates may be obtained by using information on the relationship between λ_s and λ_d from other cells. Instead of estimating λ_s and λ_d for each cell, we estimate λ_s and the odds ratio (e.g., Agresti 1984)

$$(2)\qquad \theta_d = \frac{\lambda_d(1-\lambda_s)}{\lambda_s(1-\lambda_d)}$$

in each cell. If $\theta = 1$, then $\lambda_d = \lambda_s$; and if $\theta > 1$, then $\lambda_d > \lambda_s$. The advantage of this approach is that we can test for between-cell variability in θs, and if it is not significant we could estimate a common θ and obtain estimates of λ_d in each cell using the common θ and cell-specific estimates for λ_s. The double tag reporting rate in each cell is then obtained using

$$(3)\qquad \lambda_d = \frac{\theta\lambda_s}{\theta\lambda_s + 1 - \lambda_s}$$

This procedure should provide better reporting rate estimates if we think that θ in the population does not vary between cells. Note that $\mathrm{logit}(\lambda_d) = \log(\theta) + \mathrm{logit}(\lambda_s)$, and $\log(\theta)$ is the tag type effect in the logistic reporting rate model we described earlier.

Log book method

Following the directed cod fisheries in NAFO Divisions 3KL and Subdivision 3Ps, inshore fishermen with vessels <10.7 m in length are required to complete a logbook detailing the amount of gear, catch, and dates fished. The logbook also includes a description of the tagging program and a page for fishers to write down details of any tagged fish they caught (i.e., tag number, date, location, gear type, etc). A small number of fishers have used the logbook to record and report daily information about the tagged fish they caught. We examined the logbooks received during 2001 to August 2003 to determine if any tags were recorded in the logbooks but not subsequently mailed in to collect the tag reward. If the reporting rate of fishers that record tagging information

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Table 3. Annual number of fish recaptured (single, high-reward) in each region by year cell that reporting rates are estimated for.

Region	Year							Total
	1997–1998	1999	2000	2001	2002	2003	2004	
3K_IN	15, 4	305, 57	84, 13	100, 9	77, 13	13, 1		594, 97
3L_INN	25, 10	208, 39	219, 33	500, 45	635, 97	398, 57	45, 8	2030, 289
3L_INS	35, 15	66, 18	53, 5	167, 20	48, 5	10, 2		379, 65
3Ps_PB	414, 184	1008, 329	1293, 177	1366, 87	1193, 71	1216, 168	687, 113	7177, 1129
3Ps_FB	56, 24	72, 40	138, 24	131, 14	158, 28	194, 35	105, 22	856, 187
3Ps_BB	5, 3	20, 8	14, 6	37, 4	20, 3	11, 5	16, 5	123, 34
OFF_SH	16, 5	25, 13	37, 12	47, 9	41, 7	29, 12	29, 9	224, 67
3Pn_4RS	5, 11	10, 9	7, 3	33, 8	23, 8	12, 6		90, 45

Table 4. Full model reporting rates (single, double tag; %).

Region	Year						
	1997–1998	1999	2000	2001	2002	2003	2004
3K_IN	**58, 58**	82, 85	**73, 73**	**100, 100**	72, **100**	**100, 100**	
3L_INN	64, **100**	**85, 85**	58, 70	**100, 100**	**73, 73**	**78, 78**	**62, 62**
3L_INS	74, 84	78, **100**	74, **100**	70, **100**	**87, 87**	**56, 56**	
3Ps_PB	67, 81	73, 82	90, 91	**85, 85**	90, 98	87, 90	**83, 83**
3Ps_FB	**80, 80**	63, 76	79, 99	86, **100**	**68, 68**	71, 78	**61, 61**
3Ps_BB	71, **100**	63, **100**	34, **100**	**100, 100**	**98, 98**	**36, 36**	61, 67
OFF_SH	**84, 84**	**60, 60**	72, 90	80, **100**	**100, 100**	35, **100**	**62, 62**
3Pn_4RS	17, 31	46, **100**	64, **100**	87, **100**	71, **100**	**48, 48**	

Note: Estimates at their boundary constraints, $\lambda_s \leq \lambda_d \leq 1$, are shown in bold.

and return logbooks is the same as the overall reporting rate of the fishery, then logbook data can be used to estimate reporting rates. This assumption is unlikely to be true for the Newfoundland cod fishery; however, the logbook information provides at least a partial check of the validity of our estimates.

Results

Reporting rates based on high-reward tags

The estimates of λ_s and λ_d (Table 4) for each region–year cell in Table 3 vary considerably between regions and years. The estimates for 3Ps_PB are more reliable because of the larger number of tag returns from this region. The estimates suggest that the single tag reporting rates varied between 67% and 90%. In other regions (e.g., 3Ps_BB, OFF_SH), the reporting rates seem lower than this range, although the estimates in these regions are based on far fewer returns. This formulation is referred to as model 1.

Boundary constraints, $\lambda_s \leq \lambda_d \leq 1$, are met for 62 of 106 parameter estimates (Table 4). If we view the constraints as hypotheses about the values of the parameters, then the deviance statistic between the constrained (model 1) and unconstrained (model 2) models is 31.81 (Table 5), which is not significant compared with a χ^2 distribution with 62 degrees of freedom (χ^2_{62}, $P = 0.99$). Hence, we conclude that the constrained estimates merely indicate that the population (or true) values are close to the constraints.

Many of the differences between the single and double tag reporting rates in Table 4 seem unrealistic and probably reflect the small numbers of double-tagged cod that were caught in many of the regions. We think it is reasonable that fishermen may report more doubled-tagged cod compared with single-tagged fish because of the higher reward, but we expect the odds to be approximately constant between regions and years. The deviance statistic for the hypothesis that θ is constant in all cells (model 3) is 22.12 (Table 5), which is not significant (χ^2_{52}, $P = 0.99$). Here the alternate hypothesis is $\theta \geq 1$ and is not the same in each cell. The estimate of the common θ for all cells is 1.98, which is significantly ($P < 0.05$) greater than 1. A confidence interval for θ is provided below, based on a more parsimonious model.

Table 5. Model fits.

Model	Deviance	No. of parameters
1	17 753.18	110
2	17 721.37	110
3	17 775.30	58
4	17 806.88	12
5	17 808.23	11

We assessed annual variation in the single tag reporting rates using the model logit(λ) = region effect * year effect. The * indicates that year effects are estimated seperately for each region. The year effects are constrained to sum to zero in each region. If the estimated year effects are close to zero, then this suggests that annual variations in reporting rates are small and may be ignored without bias. The statistical significance of year effects was tested separately for each region; that is, when computing test statistics in each region, year effects were included for the other regions. We also computed profile deviance confidence intervals for the year effects.

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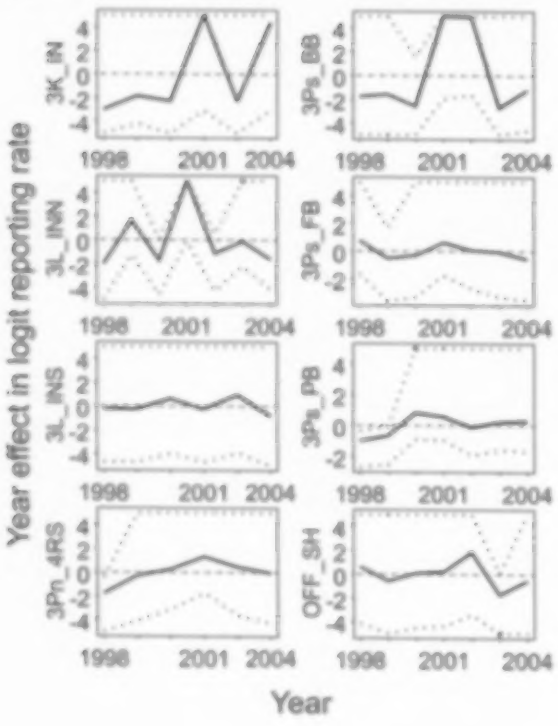
Fig. 2. Estimated year effects in logit reporting rates (solid lines), along with 95% pointwise confidence intervals (broken lines).



The basic trends in the year effects for 3L_INN and 3K_IN are similar (Fig. 2), particularly when one accounts for the pooling of information for 3K_IN in 2003 and 2004. The region effects are similar as well (Table 6). There is some statistical evidence that the year effects are not all zero in 3L_INN ($P = 0.07$), which is mostly related to the low 2000 and high 2001 estimates. The year effects are not significant in 3K_IN. Based on these results, we decided to combine reporting rates for 3K_IN and 3L_INN in all years except 2000 and 2001. The region effect for 3L_INS is also similar to 3K_IN and 3L_INN, although the trend in year effects is not. However, we decided to combine this region with 3K_IN and 3L_INN because it is in the same management area. Also, the cod fishery has been relatively small in 3L_INS during 1997–2004, so mismodeling the reporting rates for this region will not have a large effect on our estimates of combined exploitation rates for all of 3KL.

The year effects in 3PN_4RS are not significant overall (Table 6), although the negative 1997–1998 year effect is (Fig. 2). The region effect is much smaller than the other region effects. While it is plausible that the reporting rate in 1997 and 1998 in 3PN_4RS was very low, it may also be that these data are a random anomaly. There are 53 confidence intervals in Fig. 2, and we expect roughly 2–3 of these to not cover zero even when there are no year effects. Hence, we decided to simply estimate a combined reporting rate in 3PN_4RS for all years, since the overall test was not significant.

Table 6. Logit region effects estimate with 95% confidence intervals (CI), deviance test statistic (A), and P value for H_0 (i.e., year effects are zero).

Region	Estimate	CI	A	P
3K_IN	3.24	0.61, 5.00	1.41	0.92
3L_INN	2.01	0.84, 5.00	11.55	0.07
3L_INS	1.38	0.30, 5.00	0.87	0.97
3Ps_PB	1.70	1.26, 5.00	12.10	0.06
3Ps_FB	1.12	0.52, 4.10	1.81	0.94
3Ps_BB	1.67	0.02, 5.00	6.40	0.38
OFF_SH	2.18	0.21, 5.00	5.39	0.49
3PN_4RS	0.58	–0.44, 4.59	8.45	0.13

Table 7. Low-reward reporting rate (λ_0), with lower (L) and upper (U) 95% confidence points.

Region	Year	λ_0	L	U
3KL*	1997–2004	0.770	0.692	0.859
3L_INN*	2001	1†	0.871	1†
3Ps_PB + 3Ps_FB	1997–1999	0.708	0.643	0.781
3Ps_PB + 3Ps_FB	2000–2004	0.857	0.792	0.928
OFF_SH + 3Ps_BB	1997–2004	0.713	0.571	0.896
3Pn_4RS	1997–2004	0.584	0.415	0.833

*A separate rate estimated for 3L_INN in 2001.
†Upper bound (≤1).

The region effects and year effects for 3Ps_BB and OFF_SH are somewhat similar (Fig. 2; Table 6). This is not surprising because both of these areas are offshore (Fig. 1), and the cod fisheries in these two regions are prosecuted by similar gear sectors and vessel classes, although their exploitation rates may be quite different. The year effects are not significant overall in either region, so we combined reporting rates for both regions and all years.

The main effects and annual trends in reporting rates in 3Ps_FB and 3Ps_PB are also similar, apart from 1997–1998. There is evidence that year effects exist in 3Ps_PB ($P = 0.06$; Table 6). Consequently, we combined reporting rates for both regions, as well as before and after 2000. This is the simplest way we can account for the trends in 3Ps_PB (Fig. 1), although other possible approaches are described in the Discussion.

The region and year effects analyses suggested a substantial simplification of our reporting rate model (model 4; Table 5). We also found that the 2000 year effect in 3L_INN was not significant in the reduced model 4, so we removed this year effect parameter and simplified the model even more (model 5; Table 5). The full model had 106 reporting rate parameters (Table 4). The simplified model has 11 parameters (Table 5): four of these are for the tag loss rate model (described in the Tag loss rates section); six parameters are for the single-tag reporting rates (Table 7); and one odds ratio parameter (θ) is for the double tag reporting rates. The deviance statistic for the reduced model 5 compared with the full model 3 (with a common θ) is $A = 32.93$, which is not significant (χ^2_{25}, $P = 0.940$). This indicates that the simplified model also provides a reasonable fit to the data. The profile deviance confidence interval for the logit difference between the 3Ps_IN reporting rates in 1997–1999 and 2000–

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2004 is (–1.71, –0.31), which indicates that the reporting rates during 1997–1999 were significantly lower. Their 95% profile deviance confidence intervals also do not overlap (Table 7). Similarly, the increase in the 3KL reporting rate in 2001 is also significant ($P < 0.05$).

The six parameter model is our final model (Table 7). The lower 95% profile deviance confidence limit for λ_{τ} in 3KL for 2001 covers feasible reporting rates, and we conclude that the reporting rate for this region is near 1 in 2001. The estimate of θ is 1.708, with 95% confidence interval (1.223, 2.739). Hence, the odds of reporting a double-tagged fish is significantly ($P < 0.05$) higher than the odds of reporting a single-tagged fish.

Residuals similar to those in section 3 of Cadigan and Brattey (2004), but based on single and high-reward tags, do not suggest major problems with our regional and annual model for single tag reporting rates (Fig. 3). These residuals do not depend on λ_{α} or Φ and are appropriate for checking misspecifications in λ_{τ}. The average residuals tend to be close to zero, and departures are usually not significantly different from zero. We examined similarly constructed residuals for double tag returns and did not find evidence of model misspecification. We also plotted residuals against length and gear type to investigate if reporting rates depended on fish size or the type of gear used. We did not find much evidence for such dependencies.

Logbook method

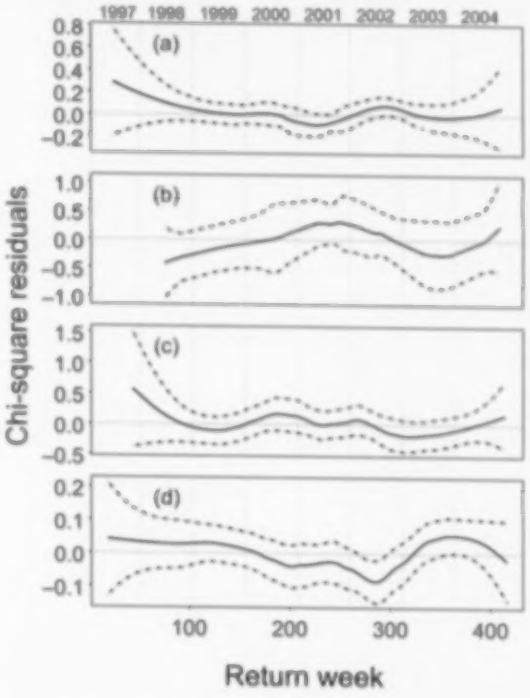
The logbooks we examined came only from fishermen in 3KL and 3Ps. The number of fishers that record the tag numbers in their logbooks is small because the number of tags received per year (Table 1) is substantially higher than the total number of logbooks received (Table 8). The logbook data represent a small fraction of the fishers that find tags. As expected, the overall percentages of tags not returned decreases as the reward value increases, from 16% and 7% for single tags ($10 reward) and double tags ($20 reward), respectively, to 5% for high-reward tags ($100 reward). These percentages agree reasonably well with the single tag reporting rate estimates (Table 7). However, a small percentage of high-reward tags (4.7%) were not returned, which indicates that our assumption of a 100% reporting rate for these tags is violated. The fishers who did not return the high-reward tags were contacted and reported that they had lost the tag before they could send it in to claim the reward. Consequently, a small adjustment in the reporting rates may be required to account for high-reward tags that are removed from captured fish but subsequently lost (see Discussion).

Tag loss rates

To utilize single tag reporting rate information in the 788 returns (4.8% of total) of single tags from double-tagged fish and to estimate double tag reporting rates, we must also estimate tag loss rates (eq. 1). In this section, we update the tag loss estimates presented in Cadigan and Brattey (2004). They used Kirkwood's (1981) tag loss model:

$$(4) \qquad \log(\Phi(t)) = b\log\left(\frac{b}{b+ct}\right), \quad b, c \geq 0$$

Fig. 3. Trends in single tag χ^2 residuals for (*a*) 3KL, (*b*) 3Pn_4RS, (*c*) OFF_SH + 3Ps_BB, and (*d*) 3Ps_PB + 3Ps_FB. The solid line represents a local linear smooth regression fit of the residuals; the broken lines represent approximated 95% confidence intervals for the trends in average residuals.



where $\Phi(t)$ is the cumulative probability that a tag is retained on a fish after time t since release. The parameters to estimate are b and c for the front and back positions, respectively. Estimates of $\Phi_1(t)$ and $\Phi_2(t)$ are presented (Fig. 4), along with 95% profile deviance confidence intervals.

Discussion

We presented an approach to accommodate for spatial and temporal variability when estimating tag reporting rates based on high-reward information. This included a novel approach to account for higher double tag reporting rates in which these rates were expressed as a function of the single tag reporting rates and an additional odds-ratio parameter. The advantage of utilizing double tag return information is increased precision of mortality rate estimates, especially when the number of double-tagged fish is large. This approach could also be adapted to deal with different reporting rates for multicomponent fisheries (Hearn et al. 2003). We described a model-fitting procedure to produce a parsimonious model for tag reporting rates for cod fisheries off Newfoundland, Canada, during 1997–2004. We advocated estimating reporting rates separately for adjacent spatial regions and temporal time periods only after a thorough statistical analysis indicated strong evidence for spatial–temporal heterogeneity in reporting rates.

The reporting rates for the substantial inshore fisheries off the south coast of Newfoundland (NAFO Subdivision 3Ps)

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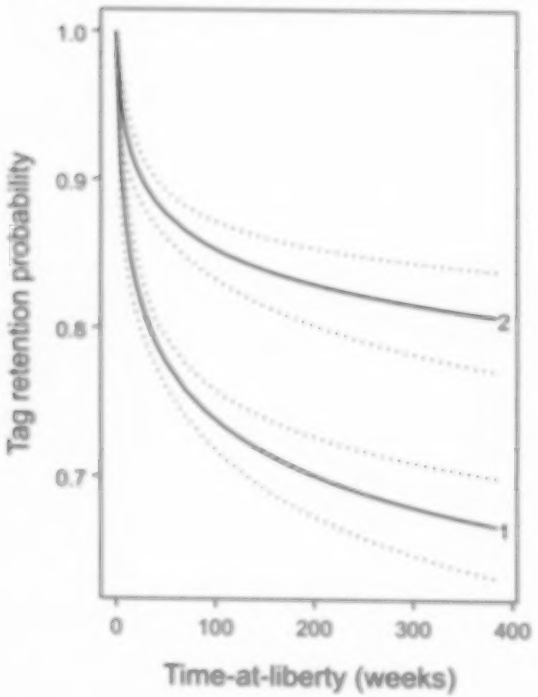
Table 8. Number of tags recorded in the <35-foot (1 ft = 0.3048 m) boat logbooks and the percent returned.

		Tags in logbook				% returned			
Year	No. of logbooks	S	DD	H	Total	S	DD	H	Total
2001	2596	57	5	0	62	74	80	—	74
2002	2143	342	9	37	388	85	100	95	86
2003	280	49	1	6	56	88	100	100	90
Total	5019	448	15	43	506	84	93	95	85

Note: S, single tags; DD, double tags; H, high-reward tags.

Fig. 4. Estimates of $\Phi_1(t)$ and $\Phi_2(t)$ using Kirkwood's parametric model (solid lines), with 95% pointwise confidence intervals (broken lines), where $\Phi_1(t)$ and $\Phi_2(t)$ are the cumulative probabilities that a tag is retained for the front and back positions, respectively, on a fish after time t since release. The right-hand numbers in the figure indicate tag position.



were higher in 2000–2004 compared with 1997–1999. This could be caused by the increasing numbers of tagged fish available to be caught by fishermen. During 1997–1999, fishermen would have generally caught smaller numbers of tagged fish compared with 2000–2004, and some fishermen may have decided that it was not financially worthwhile to return the smaller numbers of low-reward tags. Because of the annual tagging during 1997–2004, the cumulative size of the tagged populations increased, allowing higher numbers of tagged cod to be caught more recently. This may have made it financially more worthwhile to return low-reward tags. This phenomena was mentioned in Pollock et al. (2001). They also considered other ways in which the use of high-reward tags can affect low-reward tag reporting rates, both spatially and temporally. In addition, reporting rates can vary because of changes in other factors, such as management measures (e.g., quotas). This needs to be considered carefully when interpreting tag recapture information and indicates the importance of assessing for changes in reporting rates.

Annual trends in reporting rates could also be accounted for using logistic–linear models with year as a covariate. More complicated trends could be accommodated using more complicated methods such as spline regression. Such models are also straightforward to implement using our SAS code (Appendix A). However, successful implementation of such models would require more years of data than we have collected so far.

Regional or temporal variability in reporting rates in NAFO Divisions 3KL (i.e., 3L_INS, 3L_INN, and 3K_IN) was not significant ($P > 0.05$), except for 3L_INN in 2001. The reporting rate in this region and year was significantly ($P < 0.05$) higher. We do not have information that conclusively supports a cause for this increase in one year. However, the statistical evidence for the change is substantial, which is why we estimated the reporting rate separately.

There have been few direct attempts to estimate reporting rates prior to 1997 for the historically large cod fisheries off the coasts of Newfoundland and Labrador. Lear and Rice (1987) conducted interviews with inshore fishermen and estimated reporting rates to be around 72%. This is somewhat consistent with our values. Myers et al. (1997) produced estimates of tag availability parameters that were composites of reporting rates and other losses. These parameters were estimated jointly with fishing mortality rates. It is difficult to compare their estimates with the direct reporting rate estimates we have calculated. We feel our high-reward approach is preferable to the approach by Myers et al. (1996) and Myers et al. (1997) because of the common observation (Hearn et al. 1998; Hoenig et al. 1998; Pollock et al. 2001) that reporting rates estimated from a time series of standard tag-return data tend to be unreliable. Julliard et al. (2001) estimated reporting rates to be 50%–60% for some coastal cod-tagging experiments in Norway. However, as noted by Julliard et al. (2001), there are many reasons to expect the reporting rates to differ between different studies (different rewards, different advertisement, etc). This underlines the importance of continuously evaluating reporting rates using a high-reward scheme or some other method.

We feel our reporting rate estimates are more reliable than those reported in table 3 of Cadigan and Brattey (2004). They used a multiplicative model for region and year effects in reporting rates, and although they did not find significant evidence for region and year interactions, the power of their

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interaction test was probably low because the majority of tag recaptures came from one region. Their year effect estimates were probably heavily influenced by information from 3Psc (Placentia Bay), and using these year effects to infer reporting rates in other regions (i.e., 3KL) may not be appropriate. In addition, their ratio of double to single tag reporting rates did not seem reasonable in one region (3Pn_4RS; table 3 in Cadigan and Brattey 2004).

The main focus of Cadigan and Brattey (2004) was on methods to estimate tag shedding rates. They found that a model proposed by Kirkwood (1981) was suitable for their data. Our analyses of additional data to that in Cadigan and Brattey (2004) also indicated that Kirkwood's tag loss model is a good choice. The estimates of $\Phi_1(t)$ and $\Phi_2(t)$ are similar to those in Cadigan and Brattey (2004), although the confidence intervals are slightly smaller. This is to be expected because more data has been used and the model for reporting rates that we have developed here is simpler than the one used in Cadigan and Brattey (2004).

A critical assumption (e.g., Pollock et al. 2001) in our statistical model for reporting rates is that all high-reward tags are returned. Examination of information about tag reporting rates in fishermen's logbooks was useful for assessing this assumption. The logbook information agrees reasonably well with our reporting rate estimates; however, the logbooks indicated a small fraction (approximately 5%) of high-reward tags are lost. An adjustment should be made to our reporting rate estimates to account for the high-reward tags that are lost. If we fix the number of high-reward tags that are lost at 5%, then the adjusted low-reward tag reporting rates are simply the estimates in Table 7 multiplied by 0.95, although the MLE of $\lambda_{\cdot}$ for 3L_INN in 2001 may still be 1. This is a minor adjustment. Other than the fishermen's logbooks, we have very few confirmed reports of fishermen not returning a high-reward tag. Our technicians are frequently in the field and they routinely talk to fishermen. Although they do not solicit tags, the technicians are often given low-reward tags but not high-reward tags; therefore, we feel that the reporting rate for high-reward tags is close to 1.

Using more high-reward tags may improve the precision of low-reward reporting rate estimates. However, increasing the number of high-reward tags increases the cost of the reward program, which usually means that fewer fish can be tagged in total (i.e., with low and high rewards). This will increase uncertainty in fishing and natural mortality rate estimates. Pollock et al. (2001) considered this issue in more detail. Determining the most effective number of high-reward tags to apply with cod is a useful area for future research.

An important finding of our analysis is that the tag shedding rate for cod tagged at the forward position 1, which is at the base of the first dorsal fin, is higher than that at position 2, which is close to the posterior edge of the first dorsal fin. Position 1 is the standard for tagging cod. However, position 2 seems to result in better retention, and tagging in this position could reduce tag shedding from cod and lead to improved estimates of exploitation rates, etc.

We advocated conditional maximum likelihood estimation based on a multinomial likelihood function. This type of likelihood is commonly used when analyzing the results from tagging studies (e.g., Barrowman and Myers 1996; Hearn et al. 1999; Pollock et al. 2002*b*); however, these studies did not provide much motivation for the amount of conditioning that is appropriate to use. This is closely linked to the amount of pooling that is appropriate. A thorough description of the roles of conditioning in statistical inference is given by Reid (1995). For our tag-return model, the level of conditioning is determined by ξ_{xlhr}, a high-dimensional parameter, and by variations in the number of fish tagged, N_{jxl}. If the level we conditioned at involved pooling over any of the factors x, l, h, or t, then the conditional likelihood would also contain ratios of ξs and μs. This would complicate estimation of reporting rates. This is why we conditioned on $R_{\cdot\cdot lhr\cdot}$. An exception is if we could tag the same number of fish in each length class. In this situation, we could pool over lengths and yet the conditional likelihood would still involve only reporting rates and tag shedding parameters. This would involve substantial data summarization and, consequently, simplified estimation; however, such a tagging protocol would be difficult to implement and would probably result in much fewer fish tagged and released. A similar strategy of releasing the same numbers of fish in each experiment would not allow us to pool over experiments (x), because this would involve pooling over Φ_is, which we need to estimate. Conditioning on more factors than is necessary is inefficient, for reasons described later.

Hearn et al. (1999) illustrated how inappropriate pooling can cause bias in reporting rate estimates. They considered a somewhat different setting in which reporting rates were estimated by using observers to inspect part of the catch for tags. They illustrated biases that resulted when the percentage of tags in the catch varied by age or when the age composition of the catch varied between observed and unobserved fishery components. The essential problem is that it is generally appropriate to pool across ages only when exploitation rates for each component are constant across ages, which is what we discussed in the previous paragraph. In practice, exploitation rates will often depend on age. To avoid these biases, Hearn et al. (1999) did not pool over ages and used a likelihood based on conditioning on tagged and untagged catch-at-age. This is analogous to why we did not pool over length classes.

For inspected catch data, a further conditioning step may be warranted. Consider the simple example in which there is only one age class and two fishery components. The data can be represented as a 2×2 contingency table, where the first row is the tags reported in the uninspected catch component and the tags found in the inspected catch component, and the second row is the total catch in both components. The likelihood in Hearn et al. (1999) is based on conditioning on the row totals. The likelihood involves the reporting rate parameter λ and another unknown parameter (δ) for the fraction of the catch in each component. Hearn et al. (1999) estimated these parameters simultaneously, which is not difficult to do; however, adjusting for uncertainty about δ in statistical inferences about λ is a problem when the number of returned tags is small. If inferences are additionally conditioned on the column totals, then the resulting hypergeometric distribution has only one unknown parameter, λ. This distribution is arguably more suitable for inferences about λ. This is described in more detail in section 4.5.2 of Cox and Snell (1989).

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A potential disadvantage of conditional inference that is often not discussed in applications is that any information about Φs or λs in the marginal distribution of $R_{\cdot ilkr}$ is not utilized in the conditional likelihood. For our experiments, the marginal distribution of $R_{\cdot ilkr}$ contains little information about reporting rates because the μs and ξs are high-dimensional parameters; hence, the conditional distribution is both efficient and appropriate to use. However, in other applications this may not be the case. For example, when estimating reporting rates based on multiyear tagging, all of the information about reporting rates is in the marginal distribution because there is only one type of tag.

Conditioning on more factors than is necessary is inefficient because less data are used for inferences. If only single or high-reward tags are used within a factor combination, then the tag-type probability is 1 for subsequent recaptures for all values of λ. Such recaptures do not provide information about reporting rates. Using more factors means that there are more tagged fish that are uninformative about reporting rates in the conditional likelihood. For example, in earlier analyses (Cadigan and Brattey 2004), we included a factor for the different taggers that have been used in our experiments because of their possible effect on tag loss rates (i.e., Φs). However, we found that tagger effects were not important, so we have not included them in the model we use here. In addition, we have also pooled information by 3 cm length classes, whereas in earlier analyses we used 1 cm length classes. If we included tagger effects and did not pool lengths, then the total number of releases and recaptures that we could use to estimate reporting rates would decrease by 12 073 (14%) and 2295 (14%), respectively.

The models we used to estimate reporting rates are fairly simple and easy to implement, especially using the computer code provided (Appendix A). However, if the total number of double-tagged fish is very small relative to single-tagged fish, then good estimates of type S reporting rates may be obtained from a logistic regression analysis of only the single and high-reward recaptures, with $\log(N_{Sil}/N_{Hil})$ as an offset (McCullagh and Nelder 1989). In this approach, the intercept, or more generally, region and year effects are MLEs of log reporting rates. Many statistical software packages have procedures for logistic regression analyses, and these procedures can be used to assess temporal and spatial variability in reporting rates. We routinely use such a procedure as a prescreening exercise; however, the number of double-tagged fish in our data is not small, and we prefer to also use tag returns from these fish to improve the precision of our low-reward reporting rate and mortality rate estimates.

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Appendix A. Computer code.

In this section, we present some SAS/OR, SAS/STAT, and SAS/BASE software to estimate reporting rates and tag shedding rates based on the multinomial deviance function, and also eqs. 1 and 4. An input data set must be created. The data set must contain the number of returns (r) for each factor level, the time at liberty (delt), a variable that identifies the reporting rate "cell", and the number of fish released with single, double, and high-reward tags for a length class and experiment. In our example, the data set contains additional variables that define factor levels; however, these variables are not required for estimation.

We generated a very simple simulation data set in which four experiments or batches of tagged fished were released over a 2-year period. Single and double low-reward tagged fish and high-reward tagged fish were released in each experiment. For simplicity, we only considered a very artificial population consisting of two size classes, labelled 65 and 75. The fishery was prosecuted at three distinct time periods: t = 50, 100, and 150. The populations were assumed to immediately migrate equally to two regions. The exploitation rate in region 1 (μ_1) was 20% for each pulse of fishing, and the exploitation rate in region 2 (μ_2) was 10% for each pulse. The reporting rates in regions 1 and 2 were 50% and 70%, respectively, during each pulse. This was the same for single- and double-tagged fish. Fish retained tags in position 1 at the same rate according to eq. 4, with b_1 = 0.07 and c_1 = 0.05. The tag retention at position 2 was independent of tag retention at position 1 and was obtained with b_2 = 0.04 and c_2 = 0.02. Fish experienced only fishing mortality, and the survival between fisheries was $1 - \mu_i$ in regions i = 1, 2. Observed tag recaptures were randomly generated from a Poisson distribution, with mean equal to the expected tag recaptures for each fishing pulse and region.

The example data are substantially different than our tagging data because there are many returns of each tag type within factor levels in the example, whereas for our cod data there is usually (76%) only one return within a factor level and never more than 19, because the number of fish tagged for each factor level is usually small, but the number of factor levels is very large. We fixed the number of factor levels to be small in the example to limit the space required to show the data. We also increased the number of fish tagged for each factor level to get overall sample sizes that are comparable with those in our cod data set.

The following SAS code shows how to read an example data set and estimate the reporting rate and tag shedding rate parameters, as well as compute profile deviance confidence intervals. We estimated single tag reporting rates and odds ratios separately in each region and fishing pulse; however, a constant odds ratio between cells can be obtained with minor modifications of this code.

```
****** Beginning of SAS program ******;

** Step 1, read tag-recapture data **;
** variable names **
*  exp          - tagging experiment;
*  tx           - release time;
*  len          - release len class;
*  Ns           - number of single low reward tagged fish released;
*  Nd           - number of double low reward tagged fish released;
*  Nh           - number of single high reward tagged fish released;
*  region       - recapture region;
*  t            - recapture time;
*  rep_class    - unique character name for reporting rate cells;
*  delt         - time at liberty, t-tx;
*  R_x          - number of type x recaptures, x = S,D1,D2,DD, or H;

data tag_dat;
input  exp  tx  len  Ns  Nd  Nh  region $  t  rep_class $  delt
       R_S  R_D1  R_D2  R_DD  R_H;

cards;
   1999.1 20 65 5000  500  500 R1  50 R1_050  30 227 2  3 26 41
   1999.1 20 65 5000  500  500 R1 100 R1_100  80 178 3  4 16 40
   1999.1 20 65 5000  500  500 R1 150 R1_150 130 110 5  2 10 24
   1999.1 20 65 5000  500  500 R2  50 R2_050  30 149 2  3 13 23
   1999.1 20 65 5000  500  500 R2 100 R2_100  80 122 2  2  9 18
   1999.1 20 65 5000  500  500 R2 150 R2_150 130 116 1  6 13  8
   1999.1 20 75 7000  600  600 R1  50 R1_050  30 264 1  6 20 47
   1999.1 20 75 7000  600  600 R1 100 R1_100  80 210 2  2 15 25
```

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```
1999.1 20 75 7000  600  600 R1 150 R1_150 130 159 5  5  7 25
1999.1 20 75 7000  600  600 R2  50 R2_050  30 214 1  5 12 25
1999.1 20 75 7000  600  600 R2 100 R2_100  80 148 0  4 17 24
1999.1 20 75 7000  600  600 R2 150 R2_150 130 149 1  5  6 15
1999.2 40 65 3000  300  300 R1  50 R1_050  10 144 0  1 15 31
1999.2 40 65 3000  300  300 R1 100 R1_100  60  80 1  3  8 19
1999.2 40 65 3000  300  300 R1 150 R1_150 110  68 1  4  6 17
1999.2 40 65 3000  300  300 R2  50 R2_050  10 101 1  0 11 12
1999.2 40 65 3000  300  300 R2 100 R2_100  60  77 0  2  6 10
1999.2 40 65 3000  300  300 R2 150 R2_150 110  50 1  0  6  5
1999.2 40 75 5000 1000 1000 R1  50 R1_050  10 196 1  5 42 78
1999.2 40 75 5000 1000 1000 R1 100 R1_100  60 161 2  4 22 65
1999.2 40 75 5000 1000 1000 R1 150 R1_150 110 126 2 11 19 52
1999.2 40 75 5000 1000 1000 R2  50 R2_050  10 150 1  6 21 40
1999.2 40 75 5000 1000 1000 R2 100 R2_100  60 120 2  4 22 33
1999.2 40 75 5000 1000 1000 R2 150 R2_150 110 120 3  7 17 36
2000.1 70 65 1000  110  110 R1 100 R1_100  30  40 0  1  5 10
2000.1 70 65 1000  110  110 R1 150 R1_150  80  27 2  1  4  5
2000.1 70 65 1000  110  110 R2 100 R2_100  30  24 0  2  1  3
2000.1 70 65 1000  110  110 R2 150 R2_150  80  25 0  0  3  4
2000.1 70 75 5000  500  500 R1 100 R1_100  30 217 2  8 14 53
2000.1 70 75 5000  500  500 R1 150 R1_150  80 138 0  5 15 21
2000.1 70 75 5000  500  500 R2 100 R2_100  30 138 1  4 10 20
2000.1 70 75 5000  500  500 R2 150 R2_150  80 129 3  3 12 14
2000.2 90 65 7000  700  700 R1 100 R1_100  10 287 2  6 26 56
2000.2 90 65 7000  700  700 R1 150 R1_150  60 204 2  7 30 48
2000.2 90 65 7000  700  700 R2 100 R2_100  10 236 2  2 18 35
2000.2 90 65 7000  700  700 R2 150 R2_150  60 165 5  5 15 24
2000.2 90 75 3000  200  200 R1 100 R1_100  10 128 0  0  6 22
2000.2 90 75 3000  200  200 R1 150 R1_150  60  79 0  0  5  9
2000.2 90 75 3000  200  200 R2 100 R2_100  10  98 0  0  4  4
2000.2 90 75 3000  200  200 R2 150 R2_150  60  77 1  0  7 11
;
run;

** step 2, create some indicator variables, col1-col6, for reporting cells **;

proc glmmod data = tag_dat outdesign = design noprint;
  class rep_class;
  model delt = rep_class/noint;
run;

data input;
  merge tag_dat design;
run;

options ls = 85 label;

** proc nlp computes parameter estimates and profile likelihood CI's;

proc nlp data=input tech=qn out=out outest=outest cov=6;

  min dev; ** minimize the multinomial deviance;

  array parm1[6] lam1-lam6; ** single tag log reporting rates;
  array parm2[6] odds1-odds6; ** double to single reporting rate odds ratio;
  array col[6] col1-col6; ** indicators for which reporting rates to use;

  ** starting values, b1,b2,c1,c2 are for the tag shedding model;
  parms lam1-lam6=0,odds1-odds6=0,b1-b2=0.051,c1-c2=0.045;
```

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```
   ** labels, Lami_t for single tag reporting rate in region i=1,2;
   ** and fishing pulse t=50,100,150. Labels are similar for odds ratios;

   label lam1='Lam1_050' lam2='Lam1_100' lam3='Lam1_150'
         lam4='Lam2_050' lam5='Lam2_100' lam6='Lam2_150'
         odds1='Odds1_050' odds2='Odds1_100' odds3='Odds1_150'
         odds4='Odds2_050' odds5='Odds2_100' odds6='Odds2_150'
         b1='b1' b2='b2' c1='c1' c2='c2';

   ** compute CI for reporting rates and odds ratios;
   profile lam1-lam6 odds1-odds6/alpha=0.05 ffactor=1;

   bounds -5<lam1-lam6<5,0<odds1-odds6<5,b1-b2>1e-8,c1-c2>1e-8;

   lams=0;lamd=0;
   do i = 1 to 6;
    if col[i]=1 then do;
      lams = exp(parm1[i])/(1+exp(parm1[i]));
      lamd = exp(parm1[i]+parm2[i])/(1+exp(parm1[i]+parm2[i]));
    end;
   end;

   temp = b1/(b1+c1*delt);
   phi1 = temp**b1; ** position 1 tag retention;

   temp = b2/(b2+c2*delt);
   phi2 = temp**b2; ** position 2 tag retention;

   probS = lams*phi1*Ns;
   probD1 = lams*phi1*(1-phi2)*Nd;
   probD2 = lams*phi2*(1-phi1)*Nd;
   probDD = lamd*phi1*phi2*Nd;
   probH = phi1*Nh;

   den = probS+probD1+probD2+probDD+probH;
   loglike=0;

   array prob(5) probS probD1 probD2 probDD probH;
   array R(5) R_S R_D1 R_D2 R_DD R_H;
   ** create an array of predicted values for the observations;
   array ER(5) ER_S ER_D1 ER_D2 ER_DD ER_H;

   ** compute the multinomial deviance;
   dev=0;
   do i=1 to 5;
     prob[i]=prob[i]/den;
     ER[i]=R_tot*prob[i];
     if R[i]>0 then dev=dev + 2*R[i]*log(R[i]/ER[i]);
   end;
   ** that is it;
run;

data parms; ** extract estimates and CI's from output;
  set outest(where=(_TYPE_="PL_CL" or _TYPE_="PARMS"));
  keep lam1-lam6 odds1-odds6;
run;
proc transpose data=parms out=parms;
run;

data report; ** reporting rate estimates and CI's;
```

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```
  set parms;
  test=substr(_NAME_,1,3);
  if test='lam' then do;
    col1=exp(col1)/(1+exp(col1));
    col2=exp(col2)/(1+exp(col2));
    col3=exp(col3)/(1+exp(col3));
  end;
  else do;
    col1=exp(col1);col2=exp(col2);col3=exp(col3);
  end;
  rename col1=ESTM col2=CI_L col3=CL_U;
  drop test;
run;
proc print;run;

****** end of SAS program ******;
```

The profile likelihood confidence intervals can be slow to compute. The proc print output is

Obs	_NAME_	_LABEL_	ESTM	CI_L	CL_U
1	lam1	Lam1_050	0.49998	0.41478	0.607
2	lam2	Lam1_100	0.47251	0.40599	0.553
3	lam3	Lam1_150	0.49698	0.41392	0.601
4	lam4	Lam2_050	0.72185	0.56187	0.940
5	lam5	Lam2_100	0.69360	0.56488	0.861
6	lam6	Lam2_150	0.77457	0.61593	0.989
7	odds1	Odds1_050	1.26058	1.00000	2.489
8	odds2	Odds1_100	1.00000	1.00000	1.338
9	odds3	Odds1_150	1.29097	1.00000	2.701
10	odds4	Odds2_050	1.00000	1.00000	3.342
11	odds5	Odds2_100	1.00000	1.00000	2.913
12	odds6	Odds2_150	1.11574	1.00000	148.413

These are the single tag reporting rates in each region (1 or 2) and for each fishing pulse (t = 50, 100, or 150). The double tag reporting rate odds (Odds1_100, Odds2_050, and Odds2_100) are at their boundary constraints ≥ 1 (i.e., $\lambda_d \geq \lambda_s$). We generated data assuming $\lambda_d = \lambda_s$, so the boundary constraints are not necessary; however, we included them to illustrate how to use them. The tag shedding parameter estimates printed by PROC NLP are $b_1 = 0.082076$, $c_1 = 0.031808$, $b_2 = 0.079208$, and $c_2 = 0.005598$. These values are also written to the outest SAS data set.

The output and code for this paper was generated using SAS/STAT, SAS/OR, and BASE SAS software, Version 8.01 of the SAS System for Windows NT. Copyright © 1999–2000 SAS Institute Inc. SAS and all other SAS Institute Inc. product or service names are registered trademarks or trademarks of SAS Institute Inc., Cary, N.C., and indicates USA registration.

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Myosin expression levels and enzyme activity in juvenile spotted wolffish (*Anarhichas minor*) muscle: a method for monitoring growth rates

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Abstract: The activity of glycolytic enzymes and the expression levels of myosin RNA was monitored in the white muscle of juvenile spotted wolffish (*Anarhichas minor*) reared under different temperature regimes. A group of individually tagged juvenile spotted wolffish was reared for 6 months at 4, 6, 8, and 12 °C. After the rearing trial, biopsy samples were taken from white muscle of each individual and the relationship between individual growth, enzyme activity, and myosin expression was investigated. A positive relationship between the activities of two glycolytic enzymes (pyruvate kinase and lactate dehydrogenase) and individual growth rate was observed. Using real-time polymerase chain reaction (PCR) and specially developed primers for myosin mRNA and 18S rRNA for spotted wolffish, we were able to detect differences in the relative myosin expression between experimental groups, and a positive relationship between myosin expression and specific growth rates was observed. These methods may be useful as an indicator of growth rate in wild fish and a fast and reliable indicator of growth potential under culture conditions. The method also has the potential to measure differences in white muscle synthesis in fish reared under variable environmental parameters and during different life history stages.

Résumé : Nous avons suivi l'activité des enzymes glycolytiques et les niveaux d'expression de l'ARN de la myosine dans le muscle blanc de jeunes loups tachetés (*Anarhichas minor*) élevés sous différents régimes de température. Nous avons élevé un groupe de jeunes loups tachetés marqués individuellement pendant 6 mois à 4, 6, 8 ou 12 °C. Après cet élevage, nous avons fait des prélèvements expérimentaux par biopsie de muscle blanc chez chaque individu et nous avons examiné la relation entre la croissance individuelle, l'activité enzymatique et l'expression de la myosine. Il existe une relation positive entre les activités de deux enzymes glycolytiques (la pyruvate kinase et la lactate déshydrogénase) et le taux de croissance individuel. La réaction en chaîne par polymérase (PCR) en temps réel et l'utilisation de sondes spécialement mises au point pour l'ARNm de la myosine et pour l'ADNr 18S du loup tacheté nous ont permis de déceler des différences d'expression relative de la myosine entre les groupes expérimentaux; il existe, de plus, une relation positive entre l'expression de la myosine et les taux spécifiques de croissance. Ces méthodes peuvent se révéler utiles pour servir d'indicateurs du taux de croissance chez les poissons sauvages et d'indices fiables du potentiel de croissance dans des conditions d'élevage. La méthode peut aussi mesurer les différences dans la synthèse de muscle blanc chez des poissons élevés dans des conditions environnementales diverses et chez des poissons à diverses étapes de leur cycle biologique.

[Traduit par la Rédaction]

Introduction

Biologists studying fish in their natural habitats estimate growth rate using bony structures and mark–recapture studies. These methods do not estimate short-term changes in growth rate (Pelletier et al. 1995). Activity of some of the enzymes of energy metabolism has been shown to reflect growth rates in two gadoid species (Mathers et al. 1992; Pelletier et al. 1993, 1995), and measuring biological macromolecules has been hypothesized by many investigators to be a more sensitive indicator of overall fish condition than traditional morphometric variables (Chicharo et al. 1998; Beckman et al. 2000; Weber et al. 2003).

Spotted wolffish, *Anarhichas minor*, is a cold-water marine species currently looked upon with growing interest for culture in Norway, Iceland, Canada, and Chile (Foss et al.

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2004). It was identified as one of the most promising species for cold-water aquaculture in Canadian waters when evaluated against specific biotechnical criteria (Le Francois et al. 2002). The identification of low-cost and simple growth measures is of primary importance, both for biologists studying fish in their natural habitats and for commercial fish farming operations, because it enables reliable estimation of individual and cohort growth rates. Growth rate is closely related to protein synthesis and turnover (Houlihan et al. 1995), both energetically expensive processes that have to be sustained by efficient energetic metabolism and sufficient supply of amino acids. Growth processes are thus closely related to energetic capacities of fish, and the maximal growth rate could be related to these capacities. Activities of energetic metabolism enzymes pyruvate kinase (PK) and aspartate aminotransferase (AAT) have previously been related to growth rate in both common wolffish, *Anarhichas lupus* (Lamarre et al. 2004), and Atlantic cod, *Gadus morhua* (Pelletier et al. 1995). Energetic metabolism of spotted wolffish is, however, not well studied, and we propose to assess catabolic capacity by measuring three muscle enzymes representing key metabolic pathways: amino acid oxidation (AAT), glycolysis (PK), and anaerobic glycolysis (lactate dehydrogenase, LDH).

A more recent method, the real-time reverse transcriptase – polymerase chain reaction (real-time RT–PCR), will be used to relate the expression of myosin, the primary component of the muscle, to growth and metabolic enzyme activities. RT followed by PCR allows quantification of small changes in gene expression through mRNA level estimation (Pfaffl 2001). Real-time RT–PCR is used more and more as it allows the accumulation of amplified product to be detected and measured as the reaction progresses. This technique has now been used on various fish species to analyse expression of genes coding for hormones (e.g., Caelers et al. 2004; Peterson et al. 2004) and muscular proteins (Overturf and Hardy 2001). Overturf and Hardy (2001) used real-time RT–PCR to measure expression levels of myosin mRNA in rainbow trout, *Oncorhynchus mykiss*, fed on different diets. Their results suggested myosin mRNA levels to be an informative indicator of growth rate in this species. As in this study, myosin mRNA levels will be estimated using real-time RT–PCR, but a relative quantification based on the relative expression of the target gene (myosin) versus a reference gene (18S rRNA) will be used. Normalisation of the target gene with a reference gene (housekeeping gene) is generally adequate to investigate changes in gene expression given that the rate of mRNA synthesis of the reference gene is considered stable in various tissues, even under experimental procedures (Pfaffl 2001).

The following experiment was designed to investigate the interrelationship between growth, metabolic enzyme activity (EA), and myosin relative expression (MRE) in juvenile spotted wolffish.

Materials and methods

Fish stock and rearing conditions

Eggs, stripped from wild specimens caught off the eastern coast of Iceland, were fertilized in the ratio of three females to three males in the autumn of 2002 at the facilities of Hlýri ehf at Neskaupstaður, East Iceland (65°N). The fertilized eggs were spread in single layers in 10 L incubation units (Sterner, Norway), and incubation was carried out in the incubation units in 1.8 m raceways with water depths of 5–10 cm and fixed temperatures of 6–7 °C. Decaying eggs were removed daily, and antibacterial treatment (300 ppm glutaraldehyde for 5 min) was applied three times during the incubation period. The eggs were fully hatched on 7 February 2003 after ~960 degree-days. After hatching, the larvae were transferred to start-feeding units (low water level raceways) (Hansen and Falk-Petersen 2002) and immediately weaned onto a formulated dry feed for marine fish (DAN-EX 1362 1,0 GR.; Dana Feed, Horsens, Denmark). The fish were reared in shallow raceways (Øiestad 1999), subsequently feeding on larger granulates (DAN-EX 1362 1,8 GR.; Dana Feed, Horsens, Denmark) and later on 4 mm Laxá pellets (Laxá Feedmill, Akureyri, Iceland; feed specially made for halibut) until the start of the experiment. The fish were distributed randomly into eight raceways (0.4 m × 1.8 m) with water levels of 8–10 cm, providing a total volume of 0.008 m^3 (80 L) for all temperature groups. The tagged fish were reared together with untagged fish, giving a stocking density of 10 $kg \cdot m^{-2}$ at the start of the experiment.

Water flow was set to 10–15 $L \cdot min^{-1}$ for each of the experimental units, providing a current speed of 0.5–0.7 $cm \cdot s^{-1}$ in the raceways. Oxygen saturation was measured at regular intervals in the effluent water of all raceways and was never below 80%. Daily routines included adding feed to the automatic feeders, flushing deposited faeces and excess feed, and monitoring the welfare status of the fish. Light bulbs (25W) dimmed to 5% of maximum capacity supplied the raceways with low-level light during the experiment, and all groups were maintained under a constant photoperiod of 18 h light – 6 h dark. The fish were fed a formulated floating feed (Laxá Feedmill, Akureyri, Iceland) containing 52% protein, 19% fat, and 12% carbohydrate (6 mm pellet size). The feed was distributed from automatic feeders situated close to the water inlet, thus enabling the pellets to float downstream. Feed was provided in excess from automatic feeders throughout the day.

Growth study

On 6 November 2003, a subgroup within each temperature group (n = 139) was tagged intraperitoneally with Trovan® passive transponder tags and reared at 6.0 °C until the start of the experiment. No fish died during tagging. On 2 December, weight and length were recorded to the nearest 0.1 g and 0.1 cm, respectively, for all fish. The initial mean weight (± standard deviation, SD) was 71.2 (±19.6) g and did not differ significantly between raceway units (two-way nested analysis of variance, ANOVA; power, $1 - \beta > 0.6$). All growth estimates in the present study are based on individually tagged fish. All fish were anaesthetised (phenoxyethanol, 0.05 $g \cdot L^{-1}$) and weighed, and length was recorded individually every 6 weeks during the experiment. In April, the fish were treated for external parasites (formaldehyde, 500 ppm in 30 min), so the measurement interval was extended to 9 weeks in this period.

Two rearing units were kept at 6 °C, and in addition, fish were gradually transferred from the holding temperature of 6 °C to the other three experimental temperatures (4.0, 8.0,

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Table 1. Analytical parameters used for enzyme analysis of pyruvate kinase (PK), lactate deshydrogenase (LDH), and aspartate aminotransferase (AAT).

Enzyme	Substrate	Buffer solution	pH	Reference
PK	Phospho(enol)pyruvate (5 mmol·L^{-1})	Imidazole–HCl (100 mmol·L^{-1})	8	Pelletier et al. 1995
LDH	Pyruvate (0.4 mmol·L^{-1})	Imidazole–HCl (50 mmol·L^{-1})	7	Thibault et al. 1997
AAT	Aspartate (22 mmol·L^{-1})	Phosphate (100 mmol·L^{-1})	7	Pelletier et al. 1995

and 12.0 °C) over 3 days (2–4 December, 12 °C group) or over 1 day (2–3 December, 4 °C and 8 °C groups). All treatment groups consisted of two replicate rearing units. Temperatures were measured twice daily and remained within ±0.2 °C (standard error of mean, SEM) of that prescribed. The 4.0 °C group was reared under an ambient seawater regime for Neskaupstaður, with minimum temperature in March (~ 3.0 °C) and maximum temperature in June (4.5 °C). Feed was provided in excess for 6 h daily (0900–1500). On 23–24 June, white muscle biopsies were collected from each tagged fish (n = 139) using the Super-Core II adjustable instrument (MD Tech, Gainesville, Florida) and kept on dry ice until analysed. All biopsies were taken just below midsection of the dorsal fin. Skin and superficial fibres were removed from the sample before analysis.

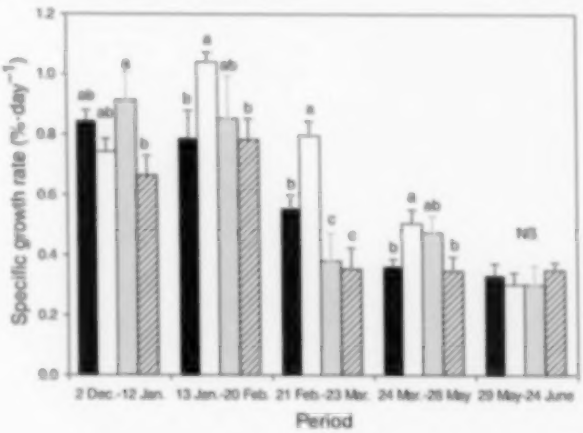
Enzymatic assays

Muscle biopsies from individually tagged fish (n = 139) were thawed on ice and homogenised in nine volumes of Tris–HCl buffer (100 mmol·L^{-1}, pH 7.5) using a Ultra Turrax T25 electrical mixer (IKA Labortechnik, Staufeni, Germany) for three 10-s periods. Between each period, samples were kept on ice for 1 min. Homogenates were centrifuged at 400*g* for 1 min. PK, LDH, and AAT were measured at 15 °C using a Lambda 40 UV/VIS spectrophotometer equipped with a thermostated cell holder (Perkin Elmer, Wellesley, Massachusetts). Analytical procedures were according to Thibault et al. (1997) for LDH and Pelletier et al. (1995) for PK and AAT (Table 1). Total protein content of muscle was determined using the bicinchoninic acid method (Smith et al. 1985). Enzyme analyses were conducted at 15 °C. All enzymatic assays were run in duplicate, and protein assays were run in triplicate.

Relative myosin expression

Total RNA was extracted from muscle biopsies (n = 139) using the TRIzol extraction method (Gibco – Life Technologies, Rockville, Maryland). Integrity and absence of DNA contaminations of the extracted RNA were verified on a 1% agarose gel stained with Sybr Green. Absence of DNA contaminations was also verified on a few samples through a "no-RT" reaction, which consisted of only the second step of the real-time RT–PCR (the PCR) and then led to no amplification in absence of DNA. RNA was then quantified using the Quant-iT™ Ribogreen® RNA reagent (Molecular Probes, Inc., Eugene, Oregon). Even with the assumption that no important DNA contaminations occurred, DNase I treatment was performed before the RT. This ensured that even imperceptible DNA contaminations would be removed and would not bias the results. Relative RT–PCR method would correct for the possible small variations in RNA content.

Fig. 1. Mean specific growth rates (G) of individually tagged spotted wolffish (*Anarhichas minor*) reared at four temperature regimes during the experimental period. Vertical whiskers indicate standard error of mean (SEM). Different letters denote significant differences (Student–Newman–Keuls test, $p << 0.05$) within each temperature regime. NS, nonsignificant. Bars: solid, 4 °C; open, 6 °C; shaded, 8 °C; hatched, 12 °C.



For each sample, RT was performed on 2 µg of total RNA in 20 µL of 1× M-MLV buffer (50 mmol·L^{-1} Tris–HCl, pH 8.3; 50 mmol·L^{-1} KCl; 3 mmol·L^{-1} $MgCl_2$; 5 mmol·L^{-1} DTT) containing 0.5 mmol·L^{-1} dNTPs mix, 2 µmol·L^{-1} oligo(dT)23 primer, 200 units M-MLV RT, and 20 units of RNase inhibitor. All products were furnished by Sigma Chemical (St. Louis, Missouri). Real-time PCR was then performed on cDNA using the iCycler IQ real-time polymerase chain reaction (PCR9 system and software as described by the manufacturer (BioRad Laboratories, Hercules, California)). Myosin mRNA contents were normalized against 18S rRNA. Primers specific to spotted wolffish for myosin mRNA and 18S rRNA were developed. Primers used for the myosin and 18S genes were as follows: forward primers: myosin-F, 5′-TGCAGGATCTGGTTGACAAGT; 18S-F, 5′-CACCACCACCGACAGAAT; reverse primers: myosin-R, 5′-TTCCTGCACTTGGACAGAAGT; 18S-R, 5′-CCTGCGGCT TAATTTGACTC.

All quantitative PCR reactions were run using the JumpStart™ Taq ReadyMix™ for quantitative PCR (Sigma-Aldrich, St. Louis, Missouri) containing all reagents except primers and templates. Conditions used for real-time PCR were as follows: 15 min at 95 °C, 35 cycles consisting of 15 s at 94 °C followed by 30 s at 60 °C and 30 s at 72 °C. Before running the samples, specificity of the real-time PCR was verified on a 6% acrylamide gel and resulted in a single product with the desired length. Efficiency of the reaction

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Table 2. Enzyme activity (units·g^{-1} tissue) and relative myosin expression (RME) of individually tagged spotted wolffish (*Anarhichas minor*), reared at four temperature regimes (4, 6, 8, and 12 °C), in relation to final weight (g), length (cm), condition factor (CF), and specific growth rate (%·day^{-1}) in the final growth period (29 May – 24 June) of the trial.

Temperature (°C)	n	Final weight (g)	Final length (cm)	Final CF	SGR (final period)	PK	AAT	LDH	RME
4	30	357.0 (108.4)b	31.5 (2.8)b	1.10 (0.11)	0.33 (0.22)	103.7 (35.9)a	4.2 (1.8)a	285.6 (78.8)a	2.56 (1.10)a
6	35	446.3 (150.4)a	34.3 (3.5)a	1.06 (0.10)	0.29 (0.24)	88.2 (32.1)b	2.1 (0.7)b	265.8 (77.2)a	1.78 (1.15)b
8	35	405.7 (161.6)ab	33.3 (3.3)a	1.05 (0.14)	0.29 (0.39)	77.8 (21.1)b	2.1 (1.0)b	202.8 (79.4)b	1.58 (1.97)b
12	39	341.2 (118.5)b	31.0 (2.8)b	1.10 (0.11)	0.35 (0.15)	85.4 (28.7)b	2.5 (1.8)b	216.9 (86.1)b	1.26 (1.35)b

Note: All values are given as mean (standard deviation). Different letters denote significant differences (Student–Newman–Keuls test, $p < 0.05$) between temperature regimes.

was also estimated for both loci with a 10-fold dilution series of six points in triplicate. Efficiencies rates and linearity of the reactions were high (efficiencies, 95.9% and 92% for 18S and myosin, respectively; Pearson's correlation coefficient > 0.99 for both loci). All samples were then run in triplicate for myosin and 18S. RME was calculated according to

$$(1) \qquad \mathrm{RME} = \frac{(E_{18S})^{CP_{18S}}}{(E_{myosin})^{CP_{myosin}}}$$

(modified from Pfaffl 2001), where E is the real-time PCR efficiency, and CP is the crossing point for each transcript (see Pfaffl 2001 for details).

Data analysis and statistical methods

Specific growth rate (G) was calculated according to the formula of Houde and Schekter (1981):

$$(2) \qquad G = (e^{g} - 1)100$$

where the instantaneous growth coefficient g is

$$(3) \qquad g = (\ln W_2 - \ln W_1)(t_2 - t_1)^{-1}$$

where W_2 and W_1 are wet weight (g) at days t_2 and t_1, respectively. Condition factor (CF) was calculated by dividing the total body weight by total body length cubed and then multiplying the result by 100.

All statistical analyses were performed with STATISTICA™ 6.0 (StatSoft Inc. 2001). Individual growth trajectories were analysed using a growth curve analysis model (GCM; Chambers and Miller 1995), which is an extension of the repeated-measures multivariate analysis of variance (MANOVA) model. The model equation of the GCM had the form

$$(4) \qquad \mathbf{Y}(n \times p) = \mathbf{X}(n \times q)\mathbf{B}(q \times p) + \mathbf{E}(n \times p)$$

where $\mathbf{Y}(n \times p)$ are the growth-at-age vectors

$$(5) \qquad \mathbf{y} = (y_1, y_2, \ldots, y_p)$$

for each p (age) measurements on n individual fish; $\mathbf{X}(n \times q)$ is the design matrix or the set of extraneous variables measured for each individual, i.e., q = age$_p$ + temperature$_i$ (i = 4, 6, 8, and 12 °C); $\mathbf{B}(q \times p)$ is the matrix of parameters estimated by the model; $\mathbf{E}(n \times p)$ is the matrix of deviations for each individual from the expected value of $\mathbf{Y} = \mathbf{XB}$.

Two-way nested analysis of covariance (ANCOVA; Zar 1984), in which replicates are nested within the temperatures and final weight is used as covariate, was applied to calculate the effect of different temperatures on specific growth rates, EA, and RME. Significant ANCOVAs were followed by a Student–Newman–Keuls multiple comparison test to locate differences among treatments (Zar 1984). When studying the relationship between growth, EA, and RME, we calculated mean growth rate for each individual and regressed those growth data against EA and RME. ANCOVA was used to test different regression lines and to verify model fit. Correlations between EA, RME, and final weight, final length, final CF, and individual growth were tested by Persons product-moment correlations.

Results

Growth

Mean individual growth trajectories were different (GCM, $\mathrm{MANOVA}_{\mathrm{GROUP}}$, Wilks' $\Lambda_{[15,517]} = 0.64$, $p < 0.001$; Fig. 1) between the four temperatures from early December onwards. Significant differences were also found in growth-at-age trajectories of the experimental groups ($\mathrm{MANOVA}_{\mathrm{GROUP\times AGE}}$, Wilks' $\Lambda_{[12,200]} = 0.74$, $p < 0.05$; Fig. 1) from early December onwards. In the first period, the fish at 8.0 °C displayed the highest growth, whereas the fish at 6.0 °C had the highest growth of all groups from January until March (Student–Newman–Keuls test, $p < 0.05$). The overall growth rate was highest at 6.0 °C (0.68%·day^{-1}) and lowest at 12.0 °C (0.48%·day^{-1}), whereas the 4.0 °C and the 8.0 °C groups had overall growth rates of 0.59% and 0.62%·day^{-1}, respectively. Final weight and final length varied between the temperatures, with higher weights and lengths at 6 °C and 8 °C (Student–Newman–Keuls test, $p < 0.05$; Table 2). Final CF did not vary between the temperature groups (Table 2).

Enzyme activity

EA differed between the experimental groups, with the 4 °C group displaying highest activity in all three enzymes (ANCOVA, $p < 0.05$), followed by the 6 °C and 12 °C groups (Table 2). There was a positive relationship between PK (Fig. 2*a*) and LDH activity (Fig. 2*c*) and individual growth rates (linear regression, $p < 0.01$). The activities of the two glycolytic enzymes tested were almost two times higher for fish with higher growth rates than for those with lower growth. However, no relationship was found between AAT activity and individual growth rates (Fig. 2*b*). Correlation between EA and final weight was found for LDH ($r = 0.29$) and PK ($r = 0.23$), and correlation between EA and final CF was found for all three enzymes (PK, $r = 0.36$; AAT, $r = 0.20$; LDH, $r = 0.38$; Fig. 3) and PK ($r = 0.37$). No relationship was found between final length and EA. The re-

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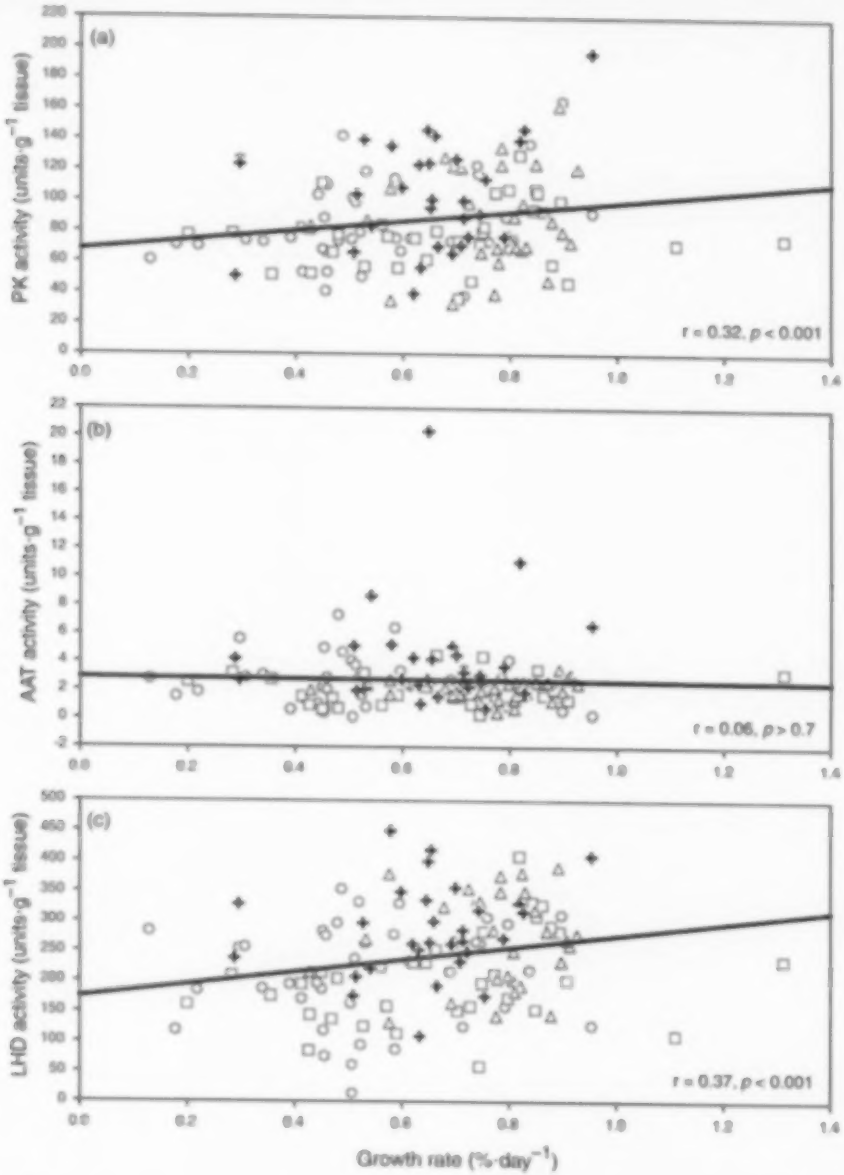
Fig. 2. Relationship between individual growth rates of juvenile spotted wolffish (*Anarhichas minor*) and activities of (*a*) pyruvate kinase (PK), (*b*) aspartate aminotransferase (AAT), and (*c*) lactate dehydrogenase (LDH). Symbols: circles, 12 °C; squares, 8 °C; triangles, 6 °C; plus signs, 4 °C.



lationship between EA and temperature varied and was stronger at 4 °C and 8 °C compared with the other temperatures. At 4 °C, a positive correlation between EA and growth was found for all three enzymes (r = 0.31, 0.34, and 0.47 for AAT, LDH, and PK, respectively, $p < 0.01$). The body weight statistic in the ANCOVAs was significant ($p < 0.05$) for all three enzymes.

Relative myosin expression

RME differed between the experimental groups, with the 4 °C group displaying highest relative difference (Student–Newman–Keuls, $p < 0.05$; Table 2), followed by the 6 °C and 8 °C groups, and the 12 °C group displaying lowest relative difference (r = 0.44; Fig. 4). No correlation between final body weight and final body length versus RME was found. In contrast, final CF was significantly correlated with RME (r = 0.20; Fig. 5). There was a positive relationship between RME and individual growth rates (ANCOVA, r = 0.44, $p < 0.001$; Fig. 3). Furthermore, myosin expression was positively related to EA for all three enzymes studied ($p < 0.05$). The relationship between growth and RME varied with temperatures, with positive correlations being found

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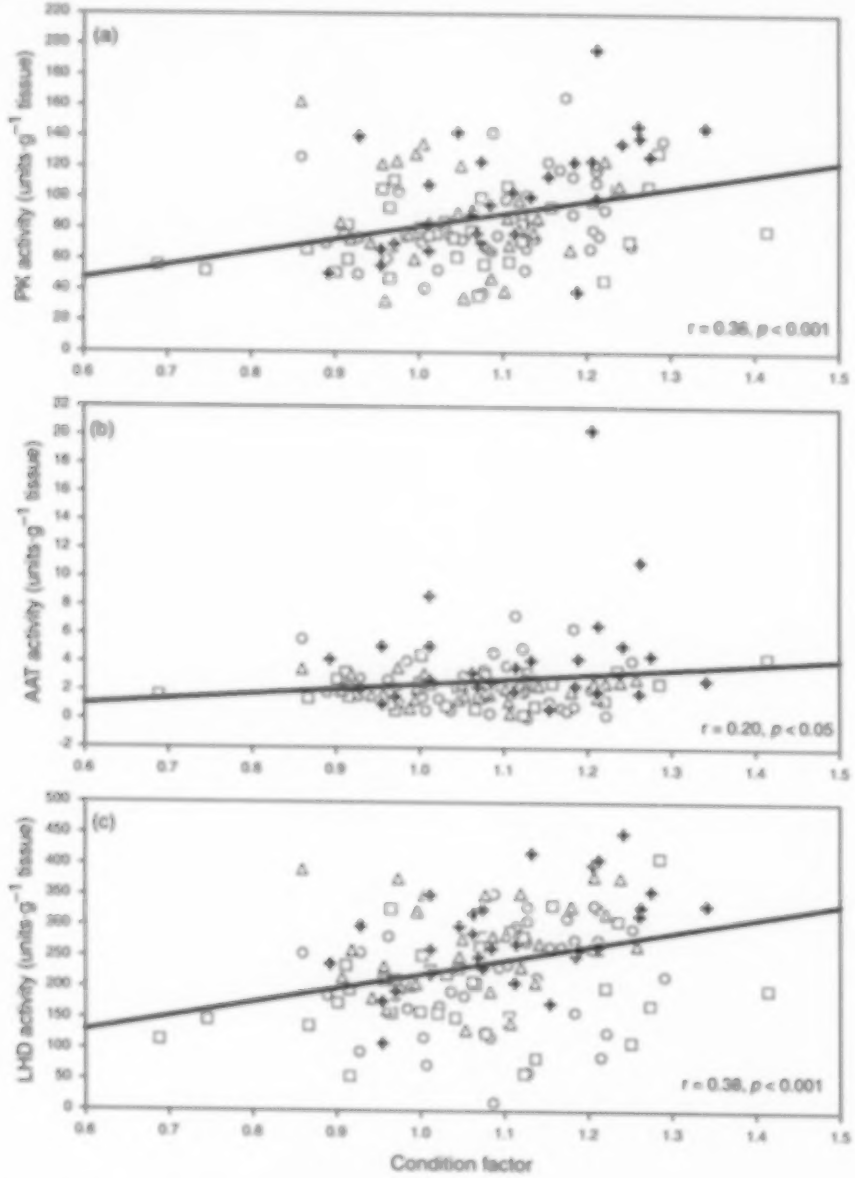
Fig. 3. Relationship between condition factor of juvenile spotted wolffish (*Anarhichas minor*) and activities of (*a*) pyruvate kinase (PK), (*b*) aspartate aminotransferase (AAT), and (*c*) lactate dehydrogenase (LDH). Symbols: circles, 12 °C; squares, 8 °C; triangles, 6 °C; plus signs, 4 °C.



at 4 °C and 8 °C. The correlation was strongest at the lowest temperature i.e., at 4 °C (r = 0.38, $p < 0.05$). The body weight statistic in the ANCOVA was significant ($F_{[1,129]}$ = 6.34, $p < 0.01$) for the myosin expression.

Discussion

Our data indicate that the levels of spotted wolffish muscle glycolytic enzymes are directly correlated with growth rates. It has previously been demonstrated in several species that the activities of glycolytic enzymes tend to increase with body size (Somero and Childress 1980). The correlation observed in our study might be size-related. However, this is contrasted with findings in Atlantic cod in which glycolytic enzyme activities in cod white muscle were shown to be size-independent (Pelletier et al. 1993). Also, our data of growth and EA were analysed using ANCOVA and thus were size-corrected using final weight as covariate. Hence, we believe that growth rate, rather than body size, is the most important factor accounting for changes in glycolytic

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Fig. 4. Relationship between individual growth rates of juvenile spotted wolffish (*Anarhichas minor*) and relative myosin expression. Symbols: circles, 12 °C; squares, 8 °C; triangles, 6 °C; plus signs, 4 °C.

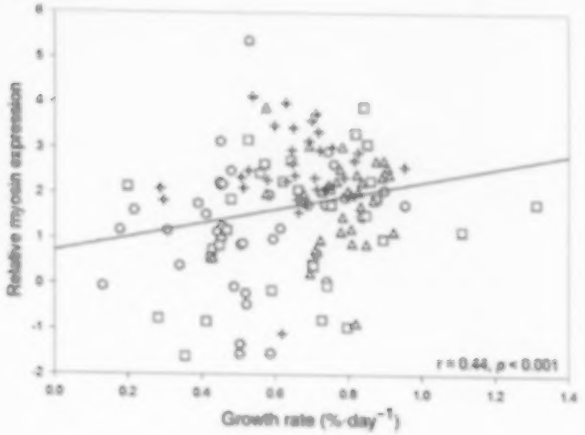
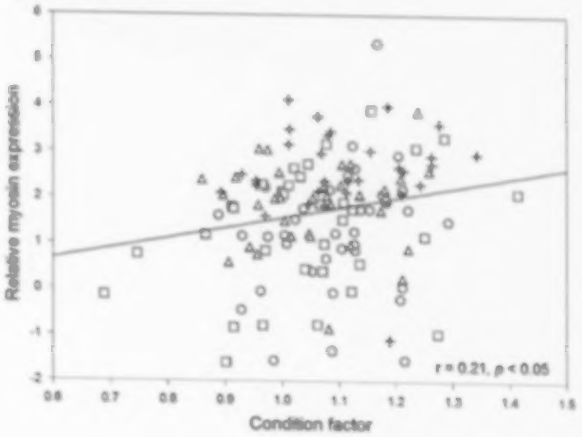


Fig. 5. Relationship between condition factor of juvenile spotted wolffish (*Anarhichas minor*) and relative myosin expression. Symbols: circles, 12 °C; squares, 8 °C; triangles, 6 °C; plus signs, 4 °C.



enzyme activities. PK and LDH activity were associated with growth rate in adult Atlantic cod (Pelletier et al. 1993, 1995). In contrast, Lamarre et al. (2004) found no clear relation between enzyme activities and growth in early juvenile (size range 0.1–2.0 g) common wolffish. The lack of relationship between EA and individual growth rate in fast-growing larvae and early juvenile (Lamarre et al. 2004) has been attributed to a reduction in the cost of protein synthesis and (or) changes in energy allocation processes (Pedersen 1997). Also, Lamarre et al. (2004) used general mean weight in their growth calculations, whereas individual mean weight was used in the present study. However, growth trajectories were related to PK, AAT, and trypsin in the data of Lamarre et al. (2004), and the authors concluded that enzymatic indicators might have some potential as a biochemical assessment of juvenile quality.

CF was related to both EA and RME in this study, whereas size (total length and weight) showed only weak or no correlation with EA and RME. Overall, mean individual growth rates and final CF showed similar relationship in setting the muscle EA and RME. Our findings agree with the original findings of Pelletier et al. (1993) on Atlantic cod in which condition (measured as seasonal effects) modified the relationship between growth and activity of the glycolytic enzymes phosphofructokinase (PFK) and pyruvate kinase (PK, included in the present study) in muscle. The study of Dutil et al. (1998) involved reversed feeding treatments of cod: one group was starved, one was fed, one was fed then starved, and the final group was starved and then fed. The conclusion of the study was that LDH activities in muscle followed condition more than recent growth rates, whereas the activity of mitochondrial enzymes in the pyloric cacae followed recent growth rates. Increased EA in fish with higher CF may reflect improved energetic status and might be used to estimate the nutritional state of wild fish. As pointed out by Lambert and Dutil (1997), CFs are inversely correlated with tissue water content and positively correlated with both specific and total energy content. However, size had only a minor effect on enzyme activities in our study, which agrees with findings in the study by Dutil et al. (1998). Although differences in size were not great, considering the range of size for this species, variability was high, which could have masked potential differences in EA and RME.

It is worth noting that the relationship between the two glycolytic enzyme (PK and LDH) activities in white muscle contrasts with the nonsignificant relationship between growth and the AAT enzyme. Through this enzyme, aspartate is transaminated to oxaloacetate, a citric acid cycle intermediate, so that surplus aspartate can be used as metabolic fuel, whereas PK and LDH are part of glycolysis. Why enzymes in different parts of the fish metabolic pathway differ in their relationship towards growth can only be speculated here, but it might be linked to variation in muscle composition. Pelletier et al. (1995) attributed the different relationships between growth and glycolytic (positive) and mitochondrial (no relationship) EAs in Atlantic cod to number, size, and shape of mitochondria in muscle tissue. Also, as proposed by Pelletier et al. (1995), increased growth rates are obviously associated with enhanced protein synthesis so that glycolytic enzymes, such as LDH and PK, may be elevated. These enzymes will accumulate in the muscle, giving the fish a higher capacity for burst swimming. This may in itself be associated with a bigger share of the food present in the environment or predator avoidance.

Myosin expression differed between the experimental groups in much the same manner as for EA. This method may thus be promising as an indicator of muscle growth in fish reared under different environmental parameters (here temperature). A similar pattern was reported by Overturf and Hardy (2001), who found myosin expression levels in muscle to vary in line with varied nutrient intake levels. However, Overturf and Hardy (2001) did not make a direct link between individual growth and myosin expression, as reported in the present study. These findings may have important consequences for both fish biologists and fish culturists as the method can give a reliable estimate of muscle synthesis and

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growth. As pointed out by Overturf and Hardy (2001), one of the most important criteria of fish growth is the monitoring of muscle (fillet) gain versus visceral fat deposition. As myosin is a major component in muscle fibres, the method would be excellent for monitoring muscle growth in both wild and cultured fish. Our data also point out an interesting relationship between myosin expression and metabolic EA: there was a significant relationship for all three enzymes and myosin expression. This further signifies that increased growth in spotted wolffish is linked to increased muscle growth and higher metabolic capacity.

It is notable that both enzyme activities and myosin expression tended to be higher at the lowest temperature (here 4 °C). In the study of Weber and Bosworth (2005), channel catfish (*Ictalurus punctatus*) reared at low temperatures had higher expression of mRNA myosin compared with fish reared at higher temperatures. It has been suggested that the prolonged exposure to low temperatures would lead to increased transcription of the gene encoding a myosin heavy chain isoform with greater ATPase activity (Guderley 1990; Weber and Bosworth 2005). The biological ramifications of increased EA and myosin expression at low temperatures remain to be determined, and further research is necessary to characterize the interrelationship of EA, RME, and temperatures. Compensation to cold temperature is generally characterized by increased cellular concentration of regulatory enzymes of glycolysis, Krebs cycle, and oxidative phosphorylation so that metabolic potential remains nearly constant all year round. This pattern of metabolic compensation has been described in many fish species in response to seasonal temperatures (Guderley 2004*a*, 2004*b*). Accordingly, EAs can be expected to be higher at 4 °C than at 6, 8, and 12 °C, indicating a higher concentration of white muscle enzymes of aerobic and anaerobic glycolysis, as well as amino acid metabolism.

Growth rate in juvenile spotted wolffish was closely related to temperature. Overall growth rate was highest at 6.0 °C. However, at 8.0 °C, growth rates were equal or only slightly lower during the later stages of the experiment, whereas the fish at 12.0 and 4.0 °C had lower overall growth rates and were significantly smaller at the termination of the experiment. This phenomenon is best explained by tradeoffs in acclimation at high and low temperatures. At low temperature, higher concentrations of metabolic enzymes are reached at the expense of reducing energy available for growth, whereas the costs of higher protein turnover at high temperature may reduce the scope for growth. When studying growth of spotted wolffish larvae up to 63 days after hatching (0.2–3.0 g), Hansen and Falk-Petersen (2002) reported a temperature effect on growth similar to that seen in this study and found maximum growth at 11.6 °C. In that study, the best growing group was moved from 12 to 10 °C and later to 8 °C. Further, Imsland et al. (2006) found optimal temperature regimes for growth in the juvenile spotted wolffish on-growing stage (100–400 g) to vary between 8 and 6 °C. On the other side of the scale, fully grown fish and broodstock are recommended to be reared at 4–6 °C (Moksness 1994; Kime and Tveiten 2002). Taken together these findings indicate a ontogenetic variation in optimum temperature for growth in spotted wolffish, with reduced temperature optimum and increased temperature tolerance with size. It has been suggested that the thermal sensitivities of anabolism and catabolism scale differently with body weight, with catabolism scaling in a stronger fashion than anabolism (Perrin (1995), but see Angilletta and Dunham (2003) and Angilletta et al. (2004)). Because growth rate is directed by the dynamic equilibrium between these two processes, the results of our study tend to reinforce the proposition of Perrin (1995).

In conclusion, the present study shows that biochemical indicators may be used to monitor and predict individual growth in juvenile spotted wolffish. These findings may have important consequences for optimization of commercial production of spotted wolffish and may be useful as an indicator of growth rate in wild spotted wolffish.

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Estimation of annual mortality rates caused by early mortality syndromes (EMS) and their impact on salmonid stock–recruit relationships

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Abstract: In this paper, we demonstrate how information from broodstocks can be combined with lab information on alevins to obtain annual stock-specific mortality estimates from early mortality syndromes (EMS) using a probabilistic approach, how a hierarchical model structure can be used to predict these mortality rates for related, partly sampled, or unsampled stocks, and why these estimates should be used to remove the effect of this mortality on stock–recruit estimates. The approach has been illustrated for Atlantic salmon (*Salmo salar*) stocks in the Baltic Sea affected by the M74 syndrome. Results indicate that data on the proportion of M74-affected females, commonly used to approximate M74 mortality, overestimate actual M74-related mortality because of a declining trend in mortality among offspring of these females. The stock-specific M74 mortality estimates are used to account for nonstationarity in the stock–recruitment relationship caused by this fluctuating mortality. Because hierarchical meta-analyses assume exchangeability, the effect of M74 mortality is removed before including these stocks within hierarchical stock–recruit analyses of Atlantic salmon stocks, which are commonly unaffected by M74 mortality. Failure to remove the effect of M74 mortality on the stock–recruit data results in underestimation of the stock's productivity and resilience to exploitation, especially in the case of stocks with steep stock–recruit curves.

Résumé : Notre travail démontre comment de l'information provenant du stock reproducteur peut être combinée par une méthode probabiliste avec des renseignements recueillis en laboratoire sur les alevins, afin d'obtenir des estimations de la mortalité due aux syndromes de mortalité précoce (EMS) et spécifique aux stocks; il montre aussi comment une structure de modèle hiérarchique peut servir à prédire ces taux de mortalité chez des stocks apparentés partiellement échantillonnés ou non échantillonnés et pourquoi ces estimations devraient être utilisées pour retirer les effets de cette mortalité dans les estimations de stock–recrutement. Nous illustrons cette méthodologie avec des stocks de saumons atlantiques (*Salmo salar*) de la Baltique affectés par le syndrome M74. Les résultats indiquent que les données sur la proportion de femelles affectées par M74, couramment utilisées pour obtenir une approximation de la mortalité due à M74, surestiment la mortalité réelle causée par M74 à cause d'une tendance décroissante de la mortalité chez les rejetons de ces femelles. Les estimations de la mortalité au M74 spécifique aux stocks servent à tenir compte de l'état non stationnaire de la relation stock–recrutement causée par cette mortalité fluctuante. Parce que les méta-analyses hiérarchiques présupposent l'échangeabilité, l'effet de la mortalité au M74 est retiré avant que les stocks ne soient inclus dans les analyses hiérarchiques de stock–recrutement des stocks de saumons atlantiques généralement non affectées par la mortalité au M74. Le fait de ne pas retirer les effets de la mortalité au M74 des données de stock–recrutement a pour effet de sous-estimer la productivité du stock et sa résilience à l'exploitation, particulièrement chez les stocks qui ont des courbes de stock–recrutement à forte pente.

[Traduit par la Rédaction]

Introduction

Stock–recruit relationships are among the most important model specifications in fisheries stock assessment, but it is often difficult to obtain the needed data to estimate the appropriate relationship. For salmonids, it is generally accepted that there is a density-dependent relationship between the spawning stock size and subsequent smolt recruitment (Jonsson et al. 1998) and that the most important effects of density dependency occur once the alevins have consumed their yolk sacs, emerged from the spawning gravel as fry, and started feeding (Elliott 1993). Traditionally, stock–

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recruit relationships require the spawning stock to be expressed in terms of eggs (Beverton and Holt 1956) because of the non-linear relationship between the number of spawners and the number of eggs, which is due to a more diverse age structure at high spawner numbers (Needle 2002). The number of eggs spawned can be estimated from information on the fecundity at age and the proportion of each age. Because stock–recruit analyses are very sensitive to errors in the measurement of the spawning stock size (Walters and Ludwig 1981), it is important to take into account any annual changes in the parameters that determine the spawning stock size. In addition, it is important to remove effects that render the stock–recruit relationship non-stationary (Hilborn and Walters 1992). Among such effects are early life history mortality syndromes, characterised by diet-related deficiencies of thiamine in broodstock and eggs and causing high mortality among yolk-sac fry and alevins (Bengtsson et al. 1999). These syndromes are an international problem, affecting various species of salmon and trout, e.g., the early mortality syndrome (EMS) in the Great Lakes, the M74 syndrome in the Baltic Sea area, and the Cayuga syndrome in the New York Finger Lakes (Fitzsimons et al. 1999). Because the mortality resulting from these syndromes occurs before density dependency takes place, i.e., before the fry emerge from the gravel, it is possible to remove the effect of this annual mortality from the estimates of the number of eggs to be used as an indicator of stock size within the stock–recruit relationship. Because the early mortality caused by these syndromes is dependent on the year, species, and location, in practise it is difficult to obtain time series estimates suitable to adjust the spawning stock size of stock–recruit data for individual stocks. Usually information on the mortality caused by these syndromes comes from observations on adult females, collected as broodstock, or on their offspring, either in hatchery rearing facilities or through lab experiments. It is therefore most common to have information on the early mortality of hatchery-reared salmonid stocks. When monitoring the yearly early life history mortality caused by these syndromes on wild stocks, budgetary concerns would only allow monitoring a limited number of stocks.

This is the first paper to estimate the effect of female-dependent early life mortality caused by EMS syndromes on stock–recruit analyses. Within this paper we demonstrate how different sources of information from broodstocks and alevins can be used to obtain annual estimates of early life mortality, how an hierarchical model approach can be used to extrapolate results from monitored salmonids stocks to unsampled stocks, and why the estimated early life history mortality rates should be taken into account in subsequent stock–recruit analyses. The approach has been illustrated for Atlantic salmon (*Salmo salar*) stock in the Baltic Sea area affected by the M74 syndrome.

Materials and methods

In this section, we first describe the available data. We then continue by describing the models, i.e., a general model for predicting the number of eggs surviving EMS and an estimation model, specific to the Baltic salmon case study, using the M74-monitoring data to provide estimates for parameters needed in the prediction model. To simplify notation, the prediction model has been developed for a single stock without specifying the age structure. The estimation model provides parameter estimates simultaneously for multiple stocks.

Data

The M74 syndrome was first detected in 1974 in Swedish Atlantic salmon hatcheries, where it caused increased mortality among alevins. The cause of the syndrome is still unknown but its occurrence has been linked to low thiamine contents within broodstock and eggs (Börjeson and Norrgren 1997; Romakkaniemi et al. 2003). The occurrence of M74 symptoms among offspring can be monitored by stripping the eggs from the female spawners and observing their development. An overview of the observed proportion of females with M74-affected offspring for different salmon stocks is provided (Table 1). Data on the occurrence of M74 are most commonly obtained when collecting broodstock for the hatcheries. As a result, most data on M74 relate to stocks that are hatchery-reared or supplemented with hatchery-reared salmon. The occurrence of the M74 syndrome has been monitored for seven hatchery-reared stocks and for five wild salmon stocks. Their location within the Baltic Sea area is shown (Fig. 1).

To relate the proportion of females with M74-affected offspring with the actual mortality among offspring, detailed information has been obtained for two wild salmon stocks, i.e., the Tornionjoki and Simojoki rivers (Table 2). By incubating in the lab batches of eggs from females collected from the wild (Vuorinen and Keinänen 1999), additional data on the total yolk-sac-fry mortality (YSFM) among offspring of individual females and on the number of females with 100% mortality among offspring have been obtained.

Prediction model

The prediction of the number of eggs, set to survive EMS, starts from the assumption that the number of females (N) contributing to the spawning stock would be known. We first assume that each female has the same chance (ρ) of its offspring being affected by EMS. Then, each female with affected offspring is assumed to have the same chance of losing all offspring (θ) to EMS. Thus, given that the chance parameters are known, the population of females can be divided into three categories, depending on the degree of EMS-related offspring mortality, using a multinomial distribution:

$$(1) \qquad (N_1, N_2, N_3) | N, \theta, \rho, \sim \text{Multinomial}(N, (1-\rho), \rho(1-\theta), \rho\theta)$$

where N_1 is the number of females with healthy offspring, and N_2 and N_3 represent the number of females with EMS-related mortality among part or all of their offspring, respectively.

If the variation in fecundity (f) among female spawners can be assumed to follow a normal distribution defined by a mean fecundity across females (μ_f) and an associated variance (σ_f^2)

$$(2) \qquad f | \mu_f, \sigma_f^2 \sim \text{Normal}(\mu_f, \sigma_f^2)$$

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Table 1. Summary of M74 data for nine different Atlantic salmon (*Salmo salar*) stocks (1985–2004), in terms of the number of females of stock *j* sampled in year *i* and with offspring affected by the M74 syndrome ($Y_{i,j}$) in comparison with the total number of females of stock *j* sampled in year *i* ($M_{i,j}$).

	Luleälven	Skellefteälven	Ume/Vindelälven	Angermanälven	Indalsälven	Ljungan	Ljusnan	Dalälven	Mörrumsån
1985	NA	NA	14/35	NA	9/219	NA	0/78	19/69	23/50
1986	NA	NA	16/82	NA	18/251	NA	0/49	4/49	24/50
1987	NA	NA	16/64	NA	20/245	NA	0/84	8/88	32/50
1988	NA	NA	12/64	NA	15/202	NA	0/75	16/79	23/50
1989	NA	NA	6/38	NA	6/192	NA	0/78	7/65	29/50
1990	NA	NA	18/59	NA	15/198	NA	0/86	4/45	39/55
1991	NA	NA	32/71	NA	14/196	NA	14/88	16/78	35/55
1992	161/279	16/40	55/71	78/157	85/190	14/22	29/89	50/63	33/60
1993	232/352	44/89	60/68	98/128	149/206	5/5	89/119	69/81	54/60
1994	269/435	54/78	146/164	52/79	148/208	6/12	105/163	70/126	4/5
1995	209/418	38/77	148/215	58/126	97/237	15/27	79/142	22/40	17/27
1996	202/392	54/70	68/87	36/57	107/167	6/22	92/128	102/178	10/18
1997	156/409	8/50	26/71	38/183	39/178	5/17	28/130	360/159	5/22
1998	22/389	2/48	6/37	3/81	2/155	2/20	7/82	14/83	NA
1999	108/316	22/53	27/51	30/108	25/126	5/20	19/46	27/82	NA
2000	67/320	7/57	27/60	29/136	27/125	1/10	29/114	36/131	NA
2001	96/322	9/51	24/62	31/122	7/100	0/10	47/102	27/82	NA
2002	119/300	8/42	20/53	56/122	25/123	6/11	23/60	56/150	NA
2003	12/270	4/60	8/53	15/120	5/128	0/2	17/100	22/164	NA
2004	10/270	0/59	2/56	4/114	0/125	NA	0/47	5/112	NA

Note: NA, not available.

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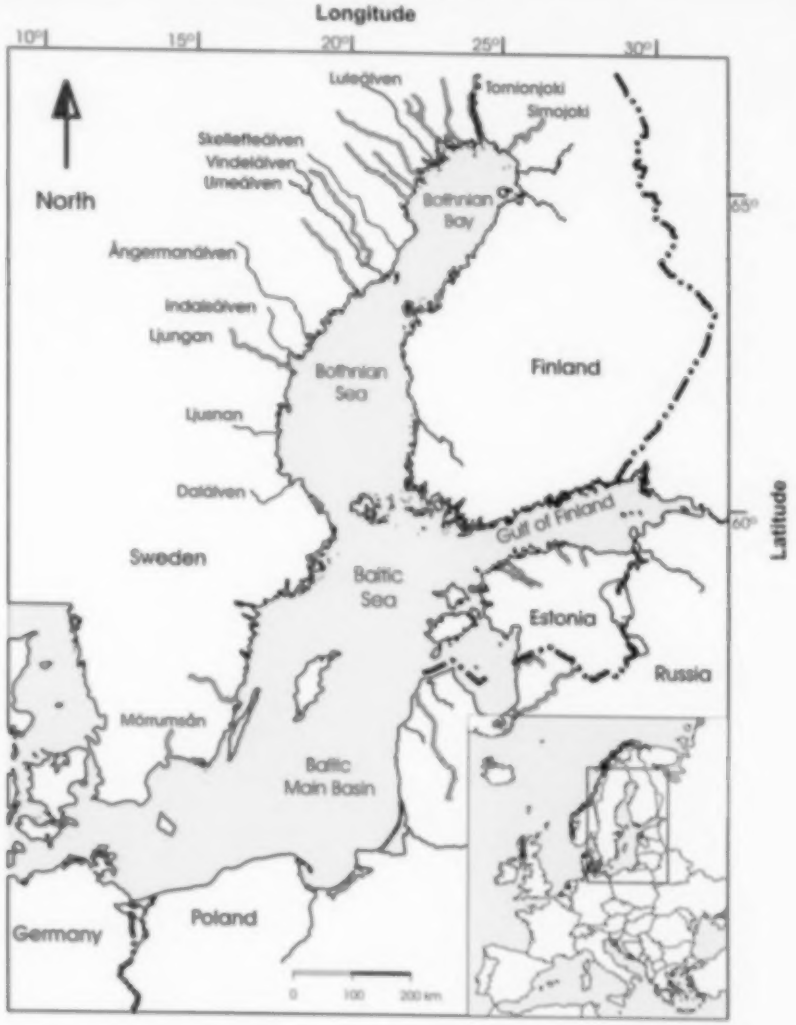
Fig. 1. Map of the Baltic Sea, indicating the location of the Atlantic salmon (*Salmo salar*) stocks included in the hierarchical analysis. Rivers containing wild salmon stocks have been indicated by thick shaded lines.



the number of eggs or offspring in each category (O_c) also has a normal distribution given the number of females in each category (N_c) and the mean and variance of the fecundity per female,

$$(3) \qquad O_c|N_c,\mu_f,\sigma_f^2 \sim \text{Normal}(\mu_f N_c, \sigma_f^2 N_c)$$

Next, the number of eggs set to die at the alevin stage as a result of EMS (D_c) needs to be calculated for each category. Clearly, no offspring will die as a result of EMS in category 1, making $D_1 = 0$. In contrast, eggs from females belonging to category 3 will all die, thus $D_3 = O_3$. However, the chance φ that an egg from a female belonging to category 2 would die at the alevin stage as a result of EMS is assumed to vary between individuals according to a beta distribution with mean μ_φ and variation parameter η_φ, whereby parameter η_φ increases as the variance decreases, and vice versa. The number of dead eggs from each female of category 2 can then be described by a beta–binomial distribution

$$(4) \qquad D_2|N_2,\mu_\varphi,\eta_\varphi,f \sim \text{Beta-Bin}(fN_2,\alpha,\beta)$$

$$(5) \qquad \alpha = \mu_\varphi\left(\frac{(\eta_\varphi+1)fN_c-(\eta_\varphi+f)}{f-1}\right)$$

$$(6) \qquad \beta = (1-\mu_\varphi)\left(\frac{(\eta_\varphi+1)fN_c-(\eta_\varphi+f)}{f-1}\right)$$

The total number of dead eggs from all females then becomes modeled as a mixture of beta–binomial (eq. 4) and normal (eq. 2) distributions (details on the derivation of these equations can be found in Appendix A). It can be seen from these equations that to predict the number of eggs dying as a result of EMS, information about the number of females and

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Table 2. Summary of M74 data for Atlantic salmon (*Salmo salar*) stocks of the Simojoki and Tornionjoki rivers (1985–2004) indicating the percentage of sampled females with offspring that display M74 symptoms (%), the total average yolk-sac-fry mortality among offspring of sampled females (%), and the percentage of sampled females with 100% mortality among offspring (%).

	Proportion of females with offspring affected by M74 (%)		Total average yolk-sac-fry mortality among offspring (%)		Proportion of females without surviving offspring (%)	
	Simojoki	Tornionjoki	Simojoki	Tornionjoki	Simojoiki	Tornionjoki
1985	0	NA	6	NA	NA	NA
1986	0	NA	2	NA	NA	NA
1987	0	NA	6	5	NA	NA
1988	0	NA	3	6	NA	NA
1989	0	NA	14	1	12	NA
1990	0	NA	4	29	NA	NA
1991	53	NA	52	70	47	NA
1992	74	NA	75	76	74	NA
1993	53	89	55	84	53	55
1994	92	76	76	66	58	49
1995	86	NA	67	NA	50	NA
1996	91	NA	71	NA	50	NA
1997	31	25	19	26	6	19
1998	59	61	54	62	38	56
1999	44	34	38	34	22	24
2000	41	41	41	35	26	21
2001	47	69	33	61	25	46
2002	7	3	2	4	0	0
2003	7	0	4	2	0	0
2004	3	NA	5	NA	3	NA

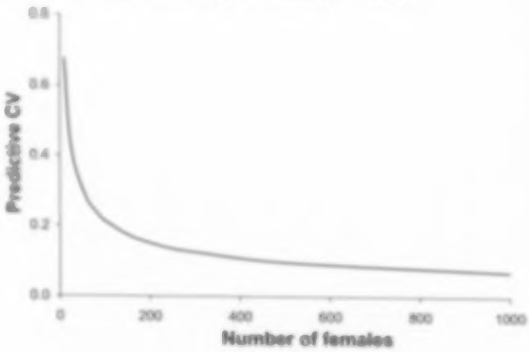
Note: NA, not available.

their fecundity is needed together with the parameters describing the occurrence and severity of the EMS-related mortality among spawner offspring. The expected value of the total number of eggs that die as a result of EMS can calculated as

$$(7)\qquad E(D_1 + D_2 + D_3) = E(D_1) + E(D_2) + E(D_3)$$
$$= \rho\mu_f N((1-\theta)\mu_\varphi + \theta)$$

The variance, however, is more difficult to compute. We used a Monte Carlo simulation to obtain the coefficient of variation (CV) of the total number of eggs set to die at the alevin stage due to EMS as a function of the number of females while keeping the other parameters fixed (Fig. 2). This illustrates that early life history mortality syndromes affecting the survival of eggs can make the estimated stock size very uncertain and the future of the population difficult to predict, especially when the population has declined to a very small number of spawners that each produce a large number of eggs.

Fig. 2. Coefficient of variation (CV) of the predictive distribution of the number of eggs that will die because of the early mortality syndrome (EMS) as a function of the number of female spawners when using the predictive model described in the text with fixed values for the EMS parameters ($\rho = 0.2$, $\theta = 0.8$, $\eta_\varphi = 20$, $\mu_\varphi = 0.3$) and fecundity values ($\mu_f = 3000$, $\sigma_f = 300$).



Estimation model

To provide information on the EMS parameters needed in the prediction model, an estimation model, specific to the Baltic salmon case study, has been developed and fitted to M74-monitoring data. Information about the number of females and their fecundity should be obtained by using other models and data sets. Following the idea of the Bayesian approach to statistical inference, we endeavor to specify the model structure based on our knowledge before obtaining the M74 data used to fit the model. We therefore base our assumptions about the syndrome on the early part of the time series (from the year 1974 when the syndrome was first found until the year 1984), after which a more detailed monitoring program was launched. The model is then fitted to time series of observations from 1985 to 2003.

A particular feature of the model proposed in this paper is the use of hierarchical structures (Gelman et al. 1995). Hierarchical structures can be used to combine data from several

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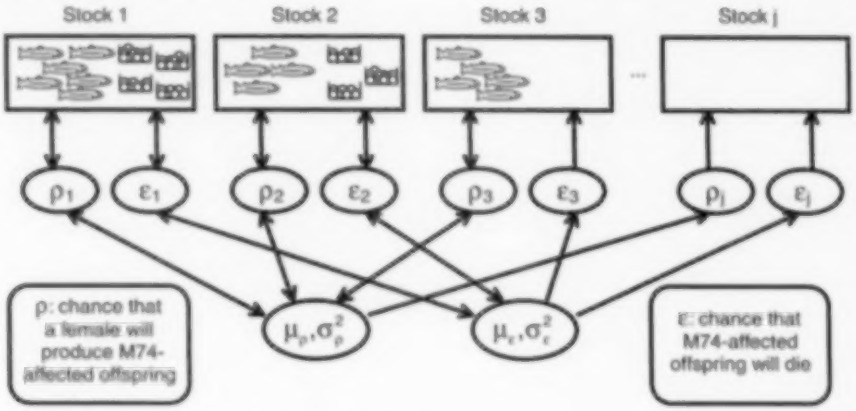
Fig. 3. Graphical presentation showing the flow of information within the hierarchical model. Information from broodstocks is used to estimate the chance that a female will produce offspring with M74 syndromes (ρ), whereas lab information is used to estimate the chance that M74-affected offspring will die (ε). Because of the transfer-of-information, the hierarchical model can predict these chances for partially sampled or unsampled stocks.



stocks allowing the estimation of model parameters both at the stock-specific level and at a higher, cross-stock level. A hierarchical model structure can be applied for a particular parameter through the use of a mean across stocks (μ) and a parameter describing the parameter variation across stocks (η). Using this model structure, it is possible to predict parameters of a stock for which no data are observed, based on the estimates across stocks and the similarities between individual stocks. The hierarchical model proposed in this paper allows obtaining stock-specific estimations for the chance that a female is affected by M74 and the chance that the offspring of an affected female would die (Fig. 3).

We define the model structure by specifying conditional probability density functions (pdfs) for all model parameters and observable quantities given their immediate parents in the directed acyclic graph (DAG; Fig. 4) (Pearl 2000). An overview of all the model parameters and their symbols is provided (Table 3). Each node in the graph is conditionally independent, given its parents, of all the other nodes that are not its descendants, and the joint density of all the variables in the graph is the product of the densities of each node conditional on its parents. Parameters without parents are given unconditional prior pdfs (Table 4).

Within the estimation model, the survival of each egg from a female is assumed to be conditionally independent of the survival of other eggs from the same female, given that the survival chance of the egg is known. Then, given that the number of sampled eggs ($N_{i,j,k}$) from individual female k and the chance of survival ($\gamma_{i,j,k}$) were known, our beliefs about the number of eggs ($X_{i,j,k}$) that survive can be described by the binomial distribution

$$(8) \quad X_{i,j,k}|\gamma_{i,j,k}, N_{i,j,k} \sim \text{Bin}(N_{i,j,k}, \gamma_{i,j,k})$$

where i denotes the year and j denotes the stock. The chance of survival is assumed to depend on the normal YSFM and the additional YSFM caused by M74. If the offspring of female k show no signs of M74, then the chance of survival depends only on the normal YSFM. Mathematically this is implemented as

$$(9) \quad \gamma_{i,j,k} = \begin{cases} \lambda_{i,j,k} & , \text{ if } I_{i,j,k} = 0 \\ \lambda_{i,j,k}\,\delta_{i,j,k}, & \text{if } I_{i,j,k} = 1 \end{cases}$$

where $\lambda_{i,j,k}$ denotes the chance to survive normal YSFM, which is assumed to be exchangeable between all individuals in every year and in every stock and modeled by

$$(10) \quad \lambda_{i,j,k}|\mu_\lambda, \eta_\lambda \sim \text{Beta}(\mu_\lambda\eta_\lambda, (1-\mu_\lambda)\eta_\lambda)$$

where μ_λ is the mean YSFM of all individuals and η_λ describes the variation between individuals. The parameter $\delta_{i,j,k}$ denotes the chance that the eggs survive M74 and $I_{i,j,k}$ denotes the occurrence of M74 symptoms among the offspring of a female

$$(11) \quad I_{i,j,k} = \begin{cases} 1, \text{ if the offspring of a female} \\ \quad \text{show M74 symptoms} \\ 0, \text{ otherwise} \end{cases}$$

All females of stock j in year i are assumed to have equal chance $\rho_{i,j}$ of producing offspring with M74 symptoms. This implies that the indicator variable $I_{i,j,k}$ follows the Bernoulli distribution

$$(12) \quad I_{i,j,k}|\rho_{i,j} \sim \text{Bern}(\rho_{i,j})$$

Further, all annual stock-specific chances $\rho_{i,j}$ are assumed to be exchangeable, i.e., there exists no prior information indicating that certain stocks are more likely to be affected by M74 than others during certain years. This means that we can assume the parameters $\rho_{i,j}$ to be a conditionally independent sample from a distribution with unknown parameters. A beta distribution is chosen to represent the distribution

$$(13) \quad \rho_{i,j}|\mu_{\rho,i}, \eta_\rho \sim \text{Beta}(\mu_{\rho,i}\eta_\rho, (1-\mu_{\rho,i})\eta_\rho)$$

where $\mu_{\rho,i}$ is the mean in year i, and η_ρ describes the amount of variation between stocks, assumed to be the same every year.

Early monitoring data indicate that the M74 syndrome can have two levels of severity: either all offspring die or part of the offspring survive. To take this phenomenon into account,

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Fig. 4. Directed acyclic graph (DAG) of the hierarchical model estimating annual early life history mortality among Atlantic salmon (*Salmo salar*) stocks in the Baltic Sea using a combination of data on the proportion of female spawners with offspring exhibiting M74 syndromes and data on the chance of survival of affected offspring. Oval nodes represent stochastic variables, square nodes represent constants, arrows represent stochastic dependencies, and double arrows indicate a logical function. A list of the symbols and their descriptions can be found in Table 3.

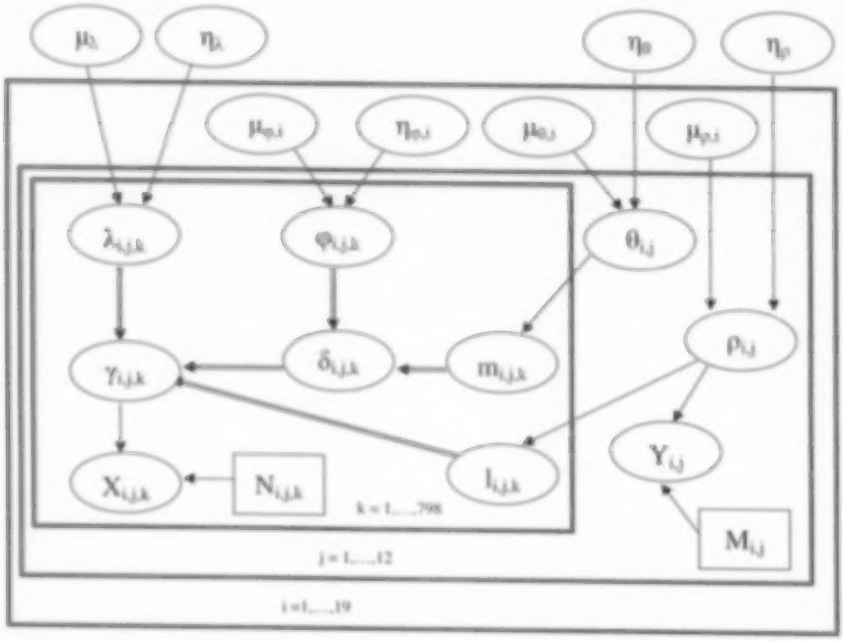

Table 3. List of symbols used within the estimation model.

Indices	
i	Year
j	Stock
k	Female
Data	
$X_{i,j,k}$	Number of surviving eggs for a sample of eggs taken in year i from female k of stock j
$N_{i,j,k}$	Total number of eggs sampled from female k of stock j in year i
$Y_{i,j}$	Number of females of stock j sampled in year i and with offspring affected by the M74 syndrome
$M_{i,j}$	Total number of females of stock j sampled in year i
Model parameters	
$I_{i,j,k}$	Indicates whether offspring from female k of stock j show symptoms of M74 in year i
$m_{i,j,k}$	Indicates whether the probability of surviving M74 mortality is equal to 0 for offspring of female k of stock j in year i
$\gamma_{i,j,k}$	Chance of an egg from female k of stock j surviving yolk-sac-fry mortality (YSFM) in year i (M74-related YSFM and "normal" YSFM)
$\lambda_{i,j,k}$	Chance of an egg from female k of stock j surviving "normal" yolk-sac-fry mortality in year i
$\delta_{i,j,k}$	Chance of an egg from female k of stock j surviving M74 mortality in year i
$\theta_{i,j}$	Chance that all offspring die, given that the offspring show M74 symptoms
$\varphi_{i,j,k}$	Chance of an egg from female k of stock j dying as a result of M74 in year i when M74 mortality of the offspring is not 100%
$\rho_{i,j}$	Chance of offspring from females of stock j dying when affected by M74 in year i
$\rho_{i,j}$	Chance that offspring from females of stock j are affected by M74 in year i

we use variable $m_{i,j,k}$ to indicate the extent at which the offspring will be affected by M74. We define

$$\delta_{i,j,k} = \begin{cases} \varphi_{i,j,k}, & \text{if } m_{i,j,k} = 0 \\ 0, & \text{if } m_{i,j,k} = 1 \end{cases} \quad (14)$$

where $\varphi_{i,j,k}$ is the chance of the offspring surviving if M74 mortality is not 100%. We assume that chance parameters $\varphi_{i,j,k}$ are exchangeable within each year. This leads to the model

$$\varphi_{i,j,k} | \mu_{\varphi,i}, \eta_{\varphi,i} \sim \text{Beta}(\mu_{\varphi,i}\eta_{\varphi,i}, (1-\mu_{\varphi,i})\eta_{\varphi,i}) \quad (15)$$

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Table 4. Prior probability density functions (pdfs) for model parameters and corresponding 95% probability interval (PI).

Parameter	Density function	95% PI
$\mu_{\rho,i}$	Beta(2,2)I(0.01,0.99)	0.09–0.9
η_ρ	Unif(2,1000)	27–975
$\mu_{\theta,i}$	Beta(2,2)I(0.05,0.95)	0.11–0.89
$\eta_{\theta\theta}$	Unif(10,1000)	12–97.5
$\mu_{\varphi,i}$	Beta(2,2)I(0.01,0.99)	0.09–0.9
$\eta_{\theta\varphi}$	Unif(2,1000)	27–975
μ_λ	Beta(2,2)I(0.01,0.99)	0.09–0.9
η_λ	Unif(2,1000)	27–975

Note: For the prior pdfs of the means, it is assumed that the values near 0 and 1 are less likely than the values in between.

where $\mu_{\theta,i}$ represents the mean across stocks and $\eta_{\theta\theta,i}$ describes the respective variation across stocks. The chance $\theta_{i,j}$ that a female with offspring exhibiting M74 symptoms will lose all offspring as a result of M74 is assumed to be equal for all females of stock j in year i and exchangeable between stocks in each year. Thus

$$m_{i,j,k} | \theta_{i,j} \sim \text{Bern}(\theta_{i,j}) \quad (16)$$

$$\theta_{i,j} | \mu_{\theta,i}, \eta_{\theta\theta} \sim \text{Beta}(\mu_{\theta,i}\eta_{\theta\theta}, (1-\mu_{\theta,i})\eta_{\theta\theta}) \quad (17)$$

where $\mu_{\theta,i}$ is the annual mean and $\eta_{\theta\theta}$ describes the variation of $\theta_{i,j}$ between stocks in every year.

In most rivers, the fate of offspring from sampled females is not recorded. Instead, data from these rivers are comprised solely of the total number of females sampled ($M_{i,j}$) and the number of females with offspring showing M74 symptoms ($Y_{i,j}$). Because

$$Y_{i,j} = \sum_{k=1}^{M_{i,j}} I_{i,j,k} \quad (18)$$

and indicators $I_{i,j,k}$ are Bernoulli distributed given $\rho_{i,j}$, the number of females with offspring showing M74 symptoms has a conditional binomial distribution

$$Y_{i,j} | M_{i,j}, \rho_{i,j} \sim \text{Bin}(M_{i,j}, \rho_{i,j}) \quad (19)$$

The constructed model allows us to use both the detailed data on M74 mortality among salmon offspring available for a few stocks and the more common, aggregated data on the number of females with offspring affected by M74-related mortality. This model construction allows us to transfer information about the chance to survive M74 ($\delta_{i,j,k}$) across stocks while still accounting for differences in severity of M74-related mortality between stocks. It is important to note that for each stock and year, data values can be given to $Y_{i,j}$ or to the pairs ($X_{i,j,k}$, $I_{i,j,k}$) but not to both at the same time.

Computation and model checking

Posterior distributions of the model parameters are not available in closed-form expressions and are therefore approximated by Markov chain Monte Carlo (MCMC) simulation (Gilks et al. 1995). The MCMC simulation is implemented using the WinBUGS 1.4 software package (Spiegelhalter et al. 2003). The convergence of the simulated Markov chains is checked using the CODA software package (Best et al. 1995). All the modeling results described in this paper have undergone tests to remove the "burn-in" (Brooks and Gelman 1998), eliminate autocorrelation, and assess convergence, and it is assumed that the reported distributions are representative of the underlying stationary distributions.

The goodness of model fit is examined by calculating mixed predictive *p*-values as proposed by Marshall and Spiegelhalter (2003). If the model fits well to the observed data set, the set of mixed predictive *p*-values should resemble a sample from a uniform distribution. This is verified by a Q–Q plot of the *p* values against the corresponding quantiles of the uniform distribution (Fig. 5).

Results

When estimating the mortality among offspring of females affected by M74, the resulting posterior pdfs indicate a decreasing trend over the years (Fig. 6). Between 1991 and 1995, on average around 80% of the offspring died because of M74 when the female was affected by M74, whereas between 1999 and 2003, this percentage had dropped to around 50%. Because information on the chance of offspring survival is available only for the Simojoki and Tornionjoki stocks, most stocks are unsampled, and the corresponding estimates rely on the estimated mean and variance for the these two stocks (Fig. 6*c*).

Combining the chance that the offspring of a female would be affected by the M74 syndrome with the chance that the offspring would die as a consequence results in the chance that an egg from that female is set to die as a result of M74 when reaching the alevin stage (Fig. 7). These estimates of the M74 mortality (%) have been compared with the data traditionally assumed to approximate M74 mortality, i.e., the percentage of females with offspring affected by M74 and the total average YSFM among offspring. In general, the percentage of females with offspring affected by M74 overestimates the M74 mortality because part of the offspring will die as a result of normal YSFM, unrelated to M74. In addition, not all offspring necessarily die when affected by M74. Because of the decreasing trend in mortality among offspring of females affected by M74 (Fig. 6), the data on proportion of females affected by M74 especially overestimate M74 mortality in recent years. For some of the stocks (e.g., Figs. 7*f* and 7*h*), the proportion of female spawners with M74-affected offspring is relatively high in 2002, whereas the estimated M74 mortality indicates a further decrease compared with previous years. Data on the total average YSFM are much better at tracking the general trend but overestimate the actual M74 mortality because these data do not distinguish between normal YSFM and YSFM caused by the M74 syndrome, i.e., the actual cause of mortality cannot be determined for each individual. In addition, these data are hard to come by given their costs.

The hierarchical model proposed in this paper allows the chance of M74 mortality among alevins of different stocks to be estimated. Because of differences in the amount of information available for each stock, the uncertainty in the M74 mortality estimates differ between stocks. The value-

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Fig. 5. Q–Q plots of the mixed predictive *p*-values for (*a*) the yolk-sac-fry mortality among offspring from individual females and (*b*) the percentage of females among the broodstock with M74 symptoms. The closer the points lie towards the 45° reference line, the better is the fit of the model to the data.

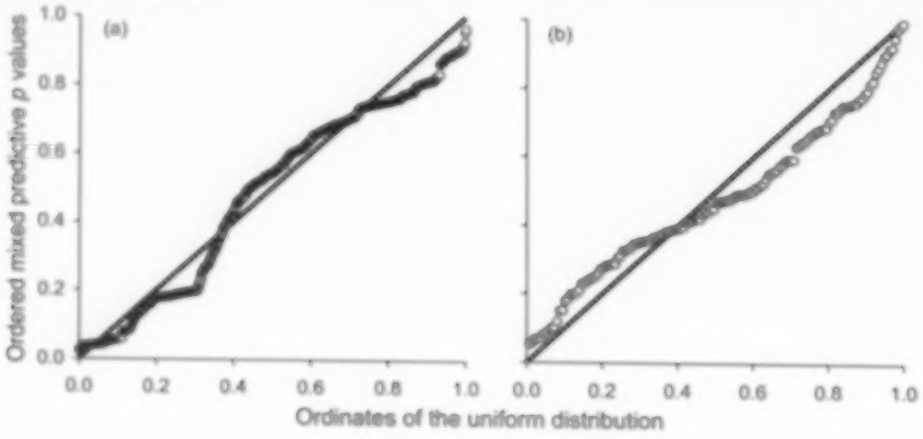
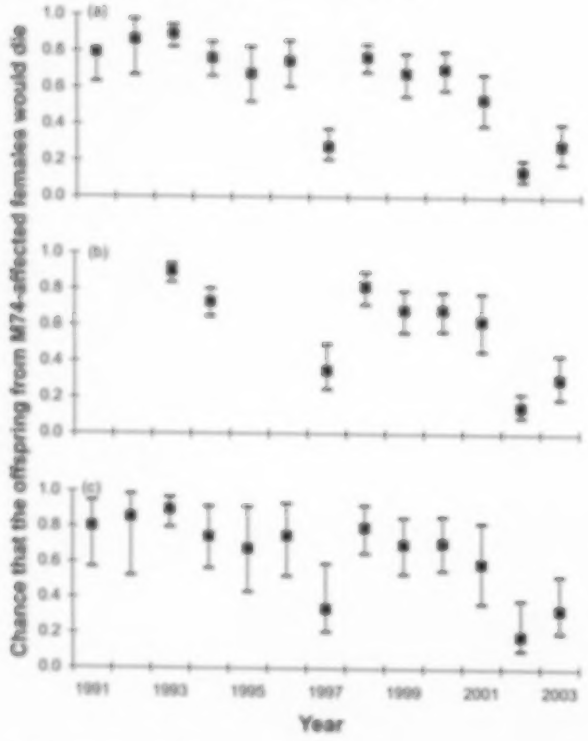


Fig. 6. Posterior probability density functions (median and 95% probability interval) for $\varepsilon_{i,j}$, i.e., the chance that M74-affected offspring would die (1991–2003): (*a*) Simojoki stock, (*b*) Tornionjoki stock, and (*c*) an unsampled Atlantic salmon (*Salmo salar*) stock within the Baltic Sea area.



of-information is illustrated for the Simojoki stock (Fig. 8). If information on the proportion of females with offspring affected by M74 is available, the posterior pdf for the M74 mortality rate becomes substantially more informative than the prior pdf. The M74 mortality estimates become even more certain if information on both the proportion of females with offspring affected by M74 and subsequent offspring mortality are available. The same figure also illustrates the transfer-of-information when using hierarchical model structures. Even when no stock-specific information is available, the hierarchical structure still allows a considerable update of the prior pdf (Fig. 8). The more stock-specific information becomes available, the less important the hierarchical structure becomes for that particular stock.

The results from the estimation model can be used within the prediction model to predict the number of eggs set to survive M74 mortality. The effect of M74 mortality on the estimated stock–recruit relationship has been illustrated for the salmon stocks in the Tornionjoki and Simojoki rivers for which stock–recruit estimates, hereafter called stock–recruit data, have been obtained by combining smolt abundance estimates (International Council for the Exploration of the Sea (ICES) 2005) with survival rate estimates from smolt to spawner stage (Michielsens et al. 2006) and fecundity estimates (ICES 2005) (Fig. 9). The resulting stock–recruit data with or without taking into account the M74 mortality have been included within an hierarchical meta-analysis of Atlantic salmon stock–recruit data. Because of the low probability of the Ricker stock–recruit function given the available Atlantic salmon stock–recruit data (Michielsens and McAllister 2004), only the Beverton–Holt stock–recruit function has been fitted to the data. The steepness parameter (z), i.e., the proportion of the long-term unfished recruitment obtained when the stock abundance is reduced to 20% of the virgin level (Mace and Doonan 1988) (Table 5), can be estimated using the hierarchical model. The steepness parameter, by definition restricted between 0.2 and 1, is greater for stocks that are more productive and resilient to exploitation. Results indicate that for the Tornionjoki stock the posterior steepness is much lower and more uncertain (mean z = 0.52, CV = 0.3) when ignoring M74 mortality in the stock–recruit data than when M74 mortality is accounted for (mean z = 0.69, CV = 0.18). For the Simojoki stock, the difference be-

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Fig. 7. Posterior probability density functions (median (solid squares) and 95% probability interval (error bars)) for the M74 mortality (%) among offspring from different Atlantic salmon (*Salmo salar*) stocks (1985–2004): (*a*) Simojoki, (*b*) Tornionjoki, (*c*) Luleälven, (*d*) Skellefteälven, (*e*) Ume/Vindelälven, (*f*) Ångermanälven, (*g*) Indalsälven, (*h*) Ljungan, (*i*) Ljusnan, (*j*) Dalälven, (*k*) Mörrumsån, and (*l*) an unsampled stock within the Baltic Sea area. This figure also shows the data assumed to approximate M74 mortality, i.e., the proportion of females with offspring affected by M74 (open diamonds) and the total average yolk-sac-fry mortality among offspring (open circles).

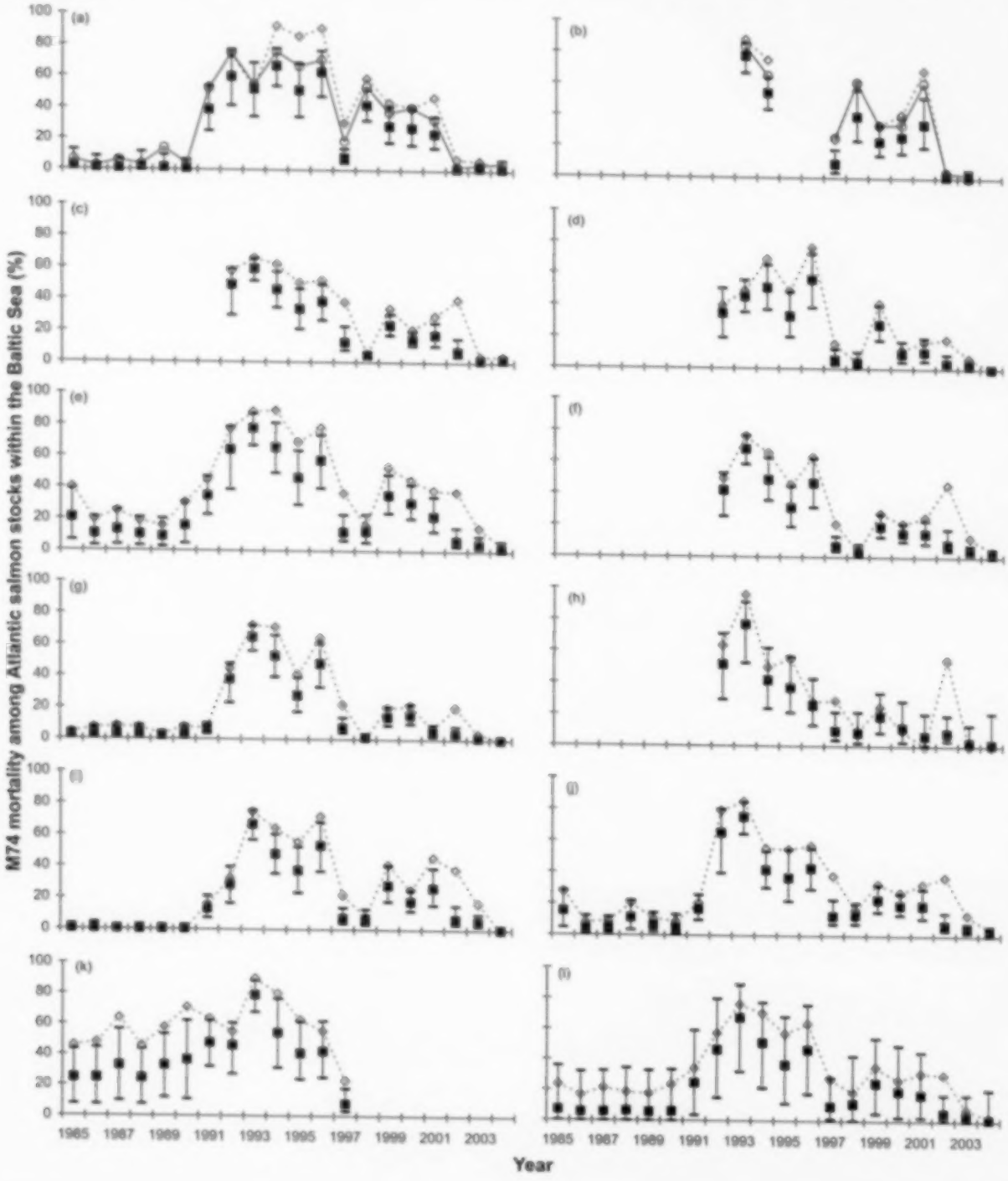

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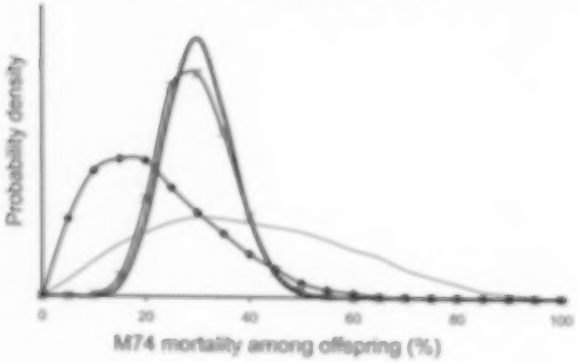
Table 5. Estimated mean and coefficient of variation (CV) for the prior and posterior probability density functions (pdfs) of the steepness for the Beverton–Holt stock–recruit function of Atlantic salmon (*Salmo salar*) stocks in the Tornionjoki and Simojoki rivers.

	Tornionjoki		Simojoki	
	Mean	CV	Mean	CV
Prior pdfs from hierarchical analysis of Atlantic salmon stock–recruit data	0.7	0.23	0.7	0.23
Posterior pdfs based on stock–recruit data with unaccounted M74 mortality	0.52	0.3	0.57	0.32
Posterior pdfs based on stock–recruit data with M74 mortality accounted for	0.69	0.18	0.59	0.28

Note: The prior pdf is obtained from a hierarchical analysis of Atlantic salmon stock–recruit data (Michielsens and McAllister 2004), whereas the posterior pdfs are obtained by including stock–recruit data, with or without accounting for M74 mortality, from these two stocks within this hierarchical analysis.

Fig. 8. Illustrative figure indicating the value-of-information for M74 data from females and (or) offspring and the transfer-of-information when using hierarchical model structures to estimate the percentage of M74 mortality among Simojoki offspring in the year 2000. Thin solid line, prior probability density function (pdf); solid diamonds, posterior pdf based on a hierarchical model structure with data from other stocks; asterisks, posterior pdf using a hierarchical model structure with M74 data of females; thick solid line, posterior pdf using a hierarchical model structure with M74 data of females and offspring.

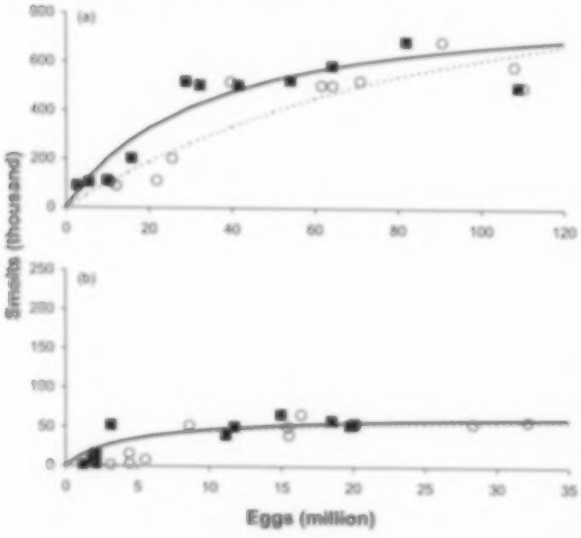


tween the results without (mean z = 0.57, CV = 0.32) or with (mean z = 0.59, CV = 0.28) accounting for M74 mortality is limited.

Discussion

Stock–recruit relationships are very important within fisheries stock assessment, even though they are very uncertain, and a large amount of effort is spent investigating this relationship (Cushing 1988). Current hierarchical methodologies for the analysis of stock–recruit data rely on the use of multiple data sets from related populations to decrease the uncertainty (Liermann and Hilborn 1997; Myers 2001; Michielsens and McAllister 2004). One major requirement when using hierarchical model structures is that the stock-specific parameters within one analysis need to be assumed exchangeable (Gelman et al. 1995). This means that there would be no information other than the stock–recruit data indicating differences in stock–recruit relationships. This renders any data series from salmonids stocks plagued by early life history mortality syndromes inappropriate for use within hierarchical analyses, even when the associated mortality is

Fig. 9. Median values of the probability density functions for the stock–recruit data of the (*a*) Tornionjoki and (*b*) Simojoki Atlantic salmon (*Salmo salar*) stocks with (■) and without (○) taking account of the early life history mortality caused by the M74 syndrome. The graphs also indicate the median values of the posterior probability distributions for the Beverton–Holt stock–recruit function obtained by including the stock–recruit data within a hierarchical stock–recruit analysis of Atlantic salmon stocks (Michielsens and McAllister 2004). To facilitate comparison between the two stocks, the Y axis of the graph for the Simojoki stock has been rescaled to represent similar egg-to-smolt ratios as for the Tornionjoki stock.



constant over the years. In addition, the extent to which salmonid populations are affected by early life history mortality syndromes often fluctuates over time, making the data series non-stationary and inappropriate when estimating the stock–recruit relationship. Because early life history mortality for salmonids can be assumed to take effect before any density dependency occurs (Elliott 1993), estimates for early life history mortality syndromes can be taken into account in stock–recruit analyses as a factor affecting stock size.

In case M74 mortality is ignored when including Baltic salmon stock–recruit data within the hierarchical meta-analysis of Atlantic salmon stocks, the resulting steepness estimates for the two Baltic salmon stocks are much lower

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than the estimates obtained by Michielsens and McAllister (2004) for the other Atlantic salmon stocks, suggesting that Atlantic salmon stocks in the Baltic Sea would have a much lower productivity and resilience to exploitation than other Atlantic salmon stocks. When accounting for M74 mortality, the steepness estimate for the Tornionjoki stock is similar to the steepness estimates of other Atlantic salmon stocks. These latter results also correspond to the biological knowledge of the productivity and resilience of this stock to exploitation. In the late 1980s, when exploitation rates were still very high (Michielsens et al. 2006), several Atlantic salmon stocks in the Baltic Sea area were close to extinction and some even completely disappeared (Romakkaniemi et al. 2003), whereas the Tornionjoki stock still produced around 60 000 wild salmon smolts annually. Once exploitation rates had decreased, wild smolt production in the Tornionjoki river increased rapidly to a current production of around one-half million wild salmon smolts, about one-third of the total wild smolt production in the Baltic Sea (ICES 2005). For the Simojoki stock, the steepness estimate remains low, even when M74 mortality is accounted for within the stock–recruit analysis. The lower steepness of this stock can be explained by the large amount of hatchery-reared salmon released within the Simojoki river to supplement the wild salmon population and aid stock recovery, thus reducing the productivity of the stock (Kallio-Nyberg and Koljonen 1997; Jutila et al. 2003). These results indicate that accounting for the mortality caused by early mortality syndromes is especially important for productive stocks.

Because stock–recruit data themselves are already very uncertain, it is important to use all available information when estimating the mortality associated with early life history mortality syndromes. Data on early life history mortality syndromes, however, are difficult to collect. In the most simplified cases, it can be estimated from spawner-related observations. As a result, the occurrence of M74 has traditionally been expressed in terms of the proportion of females with offspring affected by M74 (ICES 2005). Although initially all offspring from a female would succumb to the M74 syndrome (Börjeson and Norrgren 1997), partial M74 mortality, i.e., M74 mortality affecting only part of the offspring from one female, seems more common in recent years. This renders the occurrence of M74-related symptoms among spawner offspring no longer a suitable indicator for the actual mortality among offspring. Instead, the general information on the occurrence of M74 symptoms among offspring from individual females needs to be combined with information on the actual survival of affected offspring. Because of the cost associated with this type of research, it is not possible to do these analyses for each stock. If the stocks selected for such analyses can be considered random samples from the available stocks, a hierarchical methodology can be applied to predict the mortality among the offspring of female spawners affected by M74 for sampled and unsampled stocks.

The resulting M74 mortality estimates show a similar pattern over the years across the different stocks, with low or relatively low M74 mortality before 1991, high M74 mortality from 1992 to 1996, and decreasing M74 mortality thereafter. The decrease in M74 mortality after 1996 can potentially be explained by natural selection due to genetic differences in the survival capability of female spawners and their respective offspring (Langefors et al. 1998). Selection may therefore have reduced the proportion of females affected by the M74 syndrome in the second generation, after the high levels of M74 mortality removed a large proportion of non-M74-resistant salmon from the gene pool. In addition, differences in genetic background have been pinpointed as the cause of the differences in survival among M74-affected yolk-sac fry (Vuori et al. 2006), possibly explaining the decreasing mortality of offspring from M74-affected females in recent years.

Even though natural selection may have improved the survival probability of the population, it does not remove the actual cause of the M74 syndrome and future outbreaks are still possible. The model presented in this paper can be extended further to include explanatory variables, allowing use of the model to predict future levels of M74 mortality. Short-term prognoses about future M74 mortality can be made based on the thiamine content in the eggs (Vuorinen and Keinänen 1999). The cause of the thiamine deficiency, however, is less certain and several factors may be involved, including changes in the ecosystem and the food-web dynamics (Börjeson and Norrgren 1997). This and the fact that M74 mortality seems very low or absent in Latvian salmon stocks (ICES 2005) indicate that it might be necessary to include a spatial dimension in the model by grouping different salmon stocks depending on similar migration routes or feeding areas (Ikonen 2006). An additional hierarchical structure could then be added across different stock groups.

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Appendix A

In this appendix we explain the use of a beta–binomial distribution for predicting the number of eggs that die because of an early life history mortality syndrome (EMS), under the assumption that the survival probability varies between females.

We denote the number of females having EMS symptoms by N and the number of eggs produced by each female $i = 1,\ldots,N$ by O_i. Eggs from a female are assumed to have a female-specific probability φ_i to die because of EMS. Assuming that all eggs from one female die independently of each other, the number of dying eggs from female i, d_i, follows a binomial distribution, given that the number of eggs and the probability of dying are known, i.e.,

$$d_i|O_i,\varphi_i \sim \text{Bin}(O_i,\varphi_i)$$

Assuming that parameters φ_i are exchangeable, we can act as if they were an independent sample from some density function. Here we choose to use the beta distribution family to describe the variation between females:

$$\varphi_i|\alpha,\beta \sim \text{Beta}(\alpha,\beta)$$

with $\alpha = \mu_\varphi\eta_\varphi$ and $\beta = (1-\mu_\varphi)\eta_\varphi$. Parameter μ_φ denotes the mean across females and parameter η_φ describes the variation between females. Increasing parameter η_φ decreases the variance, and vice versa. According to standard distribution theory, the conditional distribution of d_i given parameters O_i, μ_φ, and η_φ is the mixture of beta and binomial distributions, i.e., the beta–binomial distribution

$$d_i|O_i,\mu_\varphi,\eta_\varphi \sim \text{Beta–Bin}(O_i,\mu_\varphi,\eta_\varphi)$$

The mean of a beta–binomial distribution is given by

$$E(d_i|O_i,\mu_\varphi,\eta_\varphi) = O_i\frac{\alpha}{\alpha+\beta} = O_i\mu_\varphi$$

and the variance is given by

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$$V(d_i|O_i,\mu_\varphi,\eta_\varphi) = O_i\,\frac{\alpha\beta(\alpha+\beta+O_i)}{(\alpha+\beta)^2(\alpha+\beta+1)}$$

$$= O_i\,\frac{\mu_\varphi(1-\mu_\varphi)(\eta_\varphi+O_i)}{\eta_\varphi+1}$$

Then, assuming that each female has the same number of eggs ($O_a = O_1 = O_2 = \ldots = O_N$) and that the deaths among offspring of different females are conditionally independent given parameters O_i, μ_φ, and η_φ, the mean and variance of the total number of dying eggs $D = \sum_{i=1}^{N} d_i$ can be calculated as

$$E(D|O_a,\mu_\varphi,\eta_\varphi,N) = \sum_{i=1}^{N} E(d_i|O_a,\mu_\varphi,\eta_\varphi) = NO_a\,\mu_\varphi$$

$$V(D|O_a,\mu_\varphi,\eta_\varphi,N) = \sum_{i=1}^{N} V(d_i|O_a,\mu_\varphi,\eta_\varphi,N)$$

$$= NO_a\,\frac{\mu_\varphi(1-\mu_\varphi)(\eta_\varphi+O_a)}{\eta_\varphi+1}$$

Then, by introducing a new parameter

$$\eta^* = \frac{(n_\varphi+1)O_aN-(\eta_\varphi+O_a)}{O_a-1}$$

the variance of the total number of dying eggs can be expressed also in the form

$$V(D|O_a,\mu_\varphi,\eta^*,N) = NO_a\,\frac{\mu_\varphi(1-\mu_\varphi)(\eta^*+NO_a)}{\eta^*+1}$$

which corresponds to the variance of the beta–binomial distribution

$$D|O_a,N,\alpha^*\beta^* \sim \text{Beta-Bin}(NO_a,\alpha^*,\beta^*)$$

$$\alpha^* = \mu_\varphi\left(\frac{(\eta_\varphi+1)O_aN-(\eta_\varphi+O_a)}{O_a-1}\right)$$

$$\beta^* = (1-\mu_\varphi)\left(\frac{(\eta_\varphi+1)O_aN-(\eta_\varphi+O_a)}{O_a-1}\right)$$

which gives the correct mean, variance, and range for D. Therefore, the above distribution can be used to approximate the distribution of $D = \sum_{i=1}^{N} d_i$. In simulation experiments, a beta–binomial distribution parameterised in this way has given a very close approximation for the true distribution of the sum of independent beta–binomials.

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Robust digestion and passage rate estimates for hard parts of grey seal (*Halichoerus grypus*) prey

Kate Grellier and Philip S. Hammond

Abstract: Application of digestion correction factors to measurements and counts of fish otoliths and cephalopod beaks recovered from seal scats is required before the size or quantity of prey consumed can be accurately estimated. We carried out 86 feeding trials with seven grey seals (*Halichoerus grypus*) and 18 prey species to derive estimates of digestion coefficients (to account for partial digestion), recovery rates (to account for complete digestion), and passage rates (to estimate the time between consumption and excretion of an item). Mean digestion coefficients were greatest for sandeel (*Ammodytes marinus*) and then less for large gadoid, flatfish, and *Trisopterus* spp. otoliths; and finally squid (*Loligo forbesii*) beaks. Recovery rates were greatest for squid beaks and then less for large gadoid, *Trisopterus* spp., flatfish, and sandeel otoliths. Greater than 95% of otoliths and beaks recovered were passed within 4 days (~88 h) of consumption. The large differences in partial and complete digestion rates found among prey species reinforce the importance of obtaining robust estimates of these quantities. Results from this study are the most comprehensive and systematically obtained for any species of pinniped and will allow accurate and precise estimation of the number and size of fish represented by otoliths recovered from grey seal scat samples collected in the wild.

Résumé : Il est nécessaire de corriger par un facteur qui tient compte de la digestion les mesures et les dénombrements d'otolithes de poissons et de becs de céphalopodes récupérés dans les fèces de phoques pour pouvoir estimer correctement la taille ou le nombre de proies consommées. Nous avons mené 86 essais d'alimentation avec sept phoques gris (*Halichoerus grypus*) et 18 espèces de proies afin d'obtenir les coefficients de digestion (pour tenir compte de la digestion partielle), les taux de recouvrement (pour tenir compte de la digestion complète) et les taux de passage (pour estimer le temps entre l'ingestion et l'évacuation d'un item). Les coefficients moyens de digestion sont maximaux pour les otolithes de lançons (*Ammodytes marinus*), ensuite pour ceux de grands gadoïdes, de poissons plats et de *Trisopterus* spp., enfin pour les becs de calmars (*Loligo forbesii*). Les taux de récupération sont maximaux pour les becs de calmars, puis pour les otolithes de grands gadoïdes, de *Trisopterus* spp., de poissons plats et de lançons. Plus de 95 % des otolithes et des becs récupérés ont été évacués en moins de 4 jours (~88 h) après la consommation. Les importantes différences observées dans les taux de digestion partielle et complète entre les diverses espèces de proies soulignent l'importance qu'il y a d'obtenir des estimations robustes de ces valeurs. Les résultats de notre étude représentent les données les plus complètes et celles qui ont été obtenus de la manière la plus systématique chez une espèce de pinnipède; ils permettront des estimations exactes et précises du nombre et de la taille des poissons représentés par les otolithes récupérés dans les échantillons de fèces de phoques gris prélevés en nature.

[Traduit par la Rédaction]

Introduction

There is much debate about the state of North Sea fish stocks, of which the grey seal (*Halichoerus grypus*) is a major predator, and there is great interest in obtaining current estimates of grey seal diet to assess their impact (e.g., Moore 2003). The last comprehensive estimates around Britain were made in the mid-1980s (Prime and Hammond 1990; Hammond et al. 1994*a*, 1994*b*); grey seal and fish abundances have changed substantially since then (Duck 2004; Daan et al. 2005). Estimates of grey seal diet composition and consumption of commercially important species are currently being updated using >105 000 fish otoliths and cephalopod beaks recovered from 1740 grey seal scat samples collected from around the North Sea in 2002 (Grellier 2006). The advantages and disadvantages of analysing scats to estimate seal diet composition have been much discussed. The major issues are (*i*) collection of a sufficient number of samples (addressed by Hammond and Rothery (1996)), (*ii*) nonconsumption of prey hard parts and consumption of prey with no recoverable remains (addressed by Prime and Hammond (1990) for grey seals in the North Sea), (*iii*) partial and complete digestion of hard parts as noted by Bowen (2000), and (*iv*) the potential for bias due to foraging distribution and passage rates as noted by Bowen and Harrison (1994) and Thompson et al. (1996).

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Partial and complete digestion of hard parts

Otoliths and beaks are species-specific in their shape and, for pristine specimens, there are good allometric relationships between otolith (or beak) size and fish (or cephalopod) size (see Härkönen 1986; Leopold et al. 2001; The CIAC Beak Database, available from http://www.cephbase.utmb.edu). Because hard parts from scats have passed through seals before being recovered, application of digestion correction factors is required to account for their partial or complete digestion (termed digestion coefficients and number correction factors, respectively) before the size or quantity of fish consumed can be accurately estimated. If digestion correction factors were not used, the number and size of prey consumed would be underestimated. If the biases were constant across species, the relative importance of different prey (which is how the data are usually expressed) would be accurate. However, digestion is not equal for different species; therefore, use of appropriate species-specific digestion correction factors is crucial (Bowen 2000). Furthermore, intraspecific variation in otolith digestion also occurs as a result of otoliths being subjected to different degrees of digestion. Therefore, the use of external morphological features to grade the degree of digestion (and application of appropriate grade-specific digestion coefficients) can improve estimates of prey biomass consumed (Tollit et al. 1997). Obtaining good estimates of the rate of partial and complete digestion of otoliths and beaks of different prey species is therefore an integral component of scat analysis and is one of the most important ways of improving accuracy and reducing variability in the estimated proportions of prey species in grey seal diet (Hammond and Rothery 1996).

The potential for bias due to foraging distribution and passage rates

Determining the potential for bias due to foraging distribution and passage rates is an important consideration when deciding whether scat analysis is a suitable technique for assessing the diet of a particular species in a given area. For species that spend extended periods at sea foraging several thousand kilometres away from breeding or moulting islands (e.g., southern elephant seals, *Mirounga leonina*; McConnell and Fedak 1996), scat analysis will clearly be unable to provide an accurate assessment of overall diet, although it could be used to assess diet in a limited area around haulout sites. For other species such as grey seals, scat analysis is more likely to be a suitable technique at least in areas like the North Sea where foraging trips are short (McConnell et al. 1999) and distribution at sea is relatively coastal (Matthiopoulos et al. 2004).

Prime and Hammond (1987) present the only published estimates of digestion correction factors and passage rates for grey seals and their North Sea prey. These digestion coefficients were used in previous analyses, but they are available for only a few species and are based on otolith thickness measurements, which are a poor indicator compared with length or width (Pierce et al. 1993). Recovery rates were reported for some species, but these were acknowledged to be underestimates because of loss from the system during some trials and practical difficulties throughout (Prime and Hammond 1987). Passage rates were reported only in general terms; no detail of species-specific rates was given.

The purpose of this study was to provide data to address these two outstanding issues concerning the use of scat analysis to estimate grey seal diet composition in the North Sea: (*i*) accounting for partial and complete digestion of hard parts and (*ii*) assessing the potential for bias due to foraging distribution and passage rates. Our aims were (*i*) to measure inter- and intra-individual variability in digestion and produce robust estimates of digestion coefficients and recovery rates for the major prey of British grey seals and (*ii*) to describe species-specific characteristics of the passage rate of prey hard part remains.

Materials and methods

Experimental protocol

Feeding experiments were conducted with seven adult female grey seals from August 2002 to September 2004 at the Sea Mammal Research Unit (SMRU), University of St. Andrews (Scotland). Seals were captured locally and held for up to several months before being released back into the wild. While at SMRU, the animals were housed in outdoor seawater pools at ambient temperature and offered a multi-species diet supplemented with vitamins and iron.

During feeding experiments, seals were housed individually. They had constant access to water (a pool 3 m in diameter by 1.5 m deep that held approximately 10 m^3 of seawater and was continuously flushed) and a dry area; the size of the whole enclosure was 6.20 m × 4.85 m. Overflow and outflow water passed through a 250 µm filter, collecting all debris. The recovery rate of the system was tested using a total of 2818 inert (USA Food and Drug Administration approved) coloured polystyrene beads (3 mm diameter) that were inserted into the fish offered or scattered in the pool enclosure daily. Beads were counted on recovery.

For several days prior to the start of the experiment, seals were fed either decapitated fish or species that were not going to be offered in the first few days to clear the digestive system of hard parts of interest. During experiments, seals were offered single-species meals once a day, with the exception of three occasions when two species were offered in the same meal. When this occurred, we fed fish with similar flesh (so as not to affect digestion) but dissimilar otoliths (so that there was no confusion when identifying prey remains); for example, whiting (*Merlangius merlangus*) was once fed in the same meal as poor cod (*Trisopterus minutus*). Some seals were offered the same prey species for several meals (but never on subsequent days). We aimed to keep meal size constant for each seal, but meal size did vary somewhat depending on how much of what the seals were offered was actually eaten. The pool was drained and cleaned daily within 18 h of an experimental meal being fed (average time between feeding and draining was 14 h) to ensure that we could tell the difference between egested otoliths and those that had not been ingested (information on minimum passage times was obtained from Murie and Lavigne 1986; Grellier 2006). In addition, otoliths that had not been ingested could often be distinguished from those that had been ingested by (*i*) their pristine appearance and (*ii*) using information on whether the seal ate the fish whole

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Table 1. Details of experimental prey hard parts consumed and recovered.

Common name	Scientific name	Details	Size range*	No. eaten	No. recovered	Mean rr (SE)	NCF	No. of seals	No. of trials
Norway pout	*Trisopterus esmarkii*	—	12.0–20.3	1713	1454	0.864 (0.013)	1.157	4	4
Poor cod	*Trisopterus minutus*	—	8.9–22.7	906	895	0.976 (0.023)	1.025	4	4
All trisopterans		—	8.9–22.7	2619	2349	0.920 (0.019)	1.087	5	8
Short-spined sea scorpion	*Myoxocephalus scorpius*	—	11.9–26.1	190	57	0.356 (0.069)	2.808	3	3
Dragonet	*Callionymus lyra*	—	15.0–25.5	505	192	0.306 (0.031)	3.273	3	3
Atlantic herring	*Clupea harengus*	—	20.2–29.3	1070	448	0.349 (0.041)	2.867	5	12
Atlantic mackerel	*Scomber scombrus*	—	26.6–33.0	32	23	0.719 (0.079)	1.391	1	1
Sandeel	*Ammodytes marinus*	—	13.2–22.4	3278	1151	0.350 (0.018)	2.861	5	5
Atlantic cod	*Gadus morhua*	All	15.8–51.7	193	183	0.943 (0.045)	1.060	3	10
		Small	15.8–26.5	41	39	0.951 (0.034)	1.051	1	1
		Large	34.3–51.7	152	144	0.936 (0.054)	1.069	3	9
Haddock	*Melanogrammus aeglefinus*	All	13.5–37.9	801	697	0.899 (0.029)	1.113	4	8
		Small	13.5–23.0	578	495	0.868 (0.019)	1.152	3	3
		Large	29.1–37.9	223	202	0.929 (0.036)	1.077	4	5
European hake	*Merluccius merluccius*	All	16.5–40.2	492	410	0.925 (0.014)	1.081	2	3
		Small	16.5–27.7	452	370	0.850 (0.020)	1.176	2	2
		Large	30.4–40.2	40	40	1.000 (0.000)	1.000	1	1
Whiting	*Merlangius merlangus*	All	10.0–35.0	1523	1463	0.973 (0.009)	1.027	4	7
		Small	10.0–20.7	1199	1144	0.956 (0.010)	1.046	3	3
		Large	21.0–35.0	324	319	0.991 (0.008)	1.009	4	4
All large gadoids		—	10.0–51.7	3009	2753	0.935 (0.028)	1.069	5	28
Common dab	*Limanda limanda*	—	14.8–29.3	436	376	0.816 (0.045)	1.226	4	4
Flounder	*Platichthys flesus*	—	23.1–32.5	106	72	0.705 (0.048)	1.418	2	2
Lemon sole	*Microstomus kitt*	—	14.9–32.1	462	306	0.650 (0.046)	1.539	4	4
Long rough dab	*Hippoglossoides platessoides*	—	14.0–23.9	612	550	0.860 (0.023)	1.163	2	2
European plaice	*Pleuronectes platessa*	—	13.8–34.3	519	418	0.840 (0.040)	1.190	6	8
Witch flounder	*Glyptocephalus cynoglossus*	—	24.7–32.0	257	248	0.964 (0.021)	1.037	3	3
Flounder–plaice		—	13.8–34.3	625	490	0.773 (0.044)	1.294	6	10
All flatfish		—	13.8–34.3	2392	1970	0.806 (0.039)	1.241	6	23
Squid	*Loligo forbesii*	Upper beak	13.5–337.0	375	353	0.940 (0.021)	1.064	2	3
		Lower beak	13.5–337.0	375	354	0.942 (0.021)	1.062	2	3

Note: Recovery rates from trials were combined by individual and then by prey species–size combination, prey species and finally prey type (all trisopterans, all large gadoids, flounder–plaice and all flatfish). Mean rr (SE), mean recovery rate and standard error; NCF, number correction factor.

*Fish length (cm) and squid weight (g) were measured.

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Table 2. Regressions used to infer the size of otoliths and beaks of the prey items offered.

Species	OL or LRL regression	r^2	n	OW regression	r^2	n	Reference*
Norway pout	OL = 0.436 FL + 0.028	0.98	257	OW = 0.186 FL + 0.002	0.98	257	M. Leopold, personal communication
Poor cod	OL = 0.362 FL + 1.718	0.95	267	OW = 0.178 FL + 0.731	0.93	275	M. Leopold, personal communication
Short-spined sea scorpion	OL = 0.266 FL + 0.695	0.93	368	OW = 0.129 FL + 0.355	0.93	389	M. Leopold, personal communication
Dragonet	OL = 0.112 FL + 0.763	0.94	433	OW = 0.055 FL + 0.344	0.94	481	M. Leopold, personal communication
Atlantic herring	OL = 0.154 FL + 0.386	0.96	514	OW = 0.061 FL + 0.472	0.93	541	M. Leopold, personal communication
Atlantic mackerel	OL = 0.152 FL − 0.298	0.86	71	OW = 0.040 FL + 0.323	0.73	157	M. Leopold, personal communication
Sandeel	OL = 0.184 FL − 0.642	0.79	212	OW = 0.061 FL + 0.235	0.61	212	Grellier and Hammond 2005; Grellier 2006
Atlantic cod	OL = 0.266 FL + 2.306	0.93	518	OW = 0.122 FL + 0.811	0.96	547	M. Leopold, personal communication
Haddock	OL = 0.383 FL + 1.560	0.97	450	OW = 0.137 FL + 0.703	0.96	469	M. Leopold, personal communication
European hake	OL = 0.365 FL + 1.991	0.98	60	OW = 0.131 FL + 1.046	0.96	62	M. Leopold, personal communication
Whiting	OL = 0.564 FL − 0.198	0.98	559	OW = 0.142 FL + 0.555	0.96	637	M. Leopold, personal communication
Common dab	OL = 0.179 FL + 0.734	0.97	508	OW = 0.107 FL + 0.699	0.95	513	M. Leopold, personal communication
Flounder	OL = 0.171 FL + 0.798	0.96	581	OW = 0.099 FL + 0.707	0.95	621	M. Leopold, personal communication
Lemon sole	OL = 0.091 FL + 0.624	0.87	240	OW = 0.059 FL + 0.356	0.89	240	M. Leopold, personal communication
Long rough dab	OL = 0.213 FL + 0.477	0.95	322	OW = 0.137 FL + 0.730	0.91	338	M. Leopold, personal communication
European plaice	OL = 0.203 FL + 0.486	0.99	752	OW = 0.119 FL + 0.641	0.97	787	M. Leopold, personal communication
Witch flounder	OL = 0.114 FL + 1.602	0.89	81	—	—	—	T. Härkönen, personal communication
Squid	LRL = 0.435 $W^{0.347}$	0.77	193	—	—	—	The CIAC Beak Database (http://www.cephbase.utmb.edu)

Note: Otolith length (OL), otolith width (OW), and lower rostral length (LRL) were measured in mm; fish length (FL) was measured in cm; squid weight (W) was measured in g.

*Original data provided by M. Leopold (Alterra, P.O. Box 47, 6700 AA Wageningen, the Netherlands) and T. Härkönen (Swedish Museum of Natural History, Box 50007, 104 05 Stockholm, Sweden) are summarized in Leopold et al. (2001) and Härkönen (1986), respectively.

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(i.e., whether ingestion was observed). All debris was collected after draining and cleaning and was sieved using a nest of sieves of mesh sizes 1 mm, 600 µm, and 335 µm.

In total, 18 fish and cephalopod prey species were offered (the species and sizes offered are shown in Table 1). Prey of known size (fish length was measured to the nearest 0.1 cm; squid weight was measured to the nearest 0.5 g) were offered whole or gutted (those that were obtained commercially had been gutted prior to delivery). The size of their otoliths or beaks was inferred using the relationships shown in Table 2. These species were chosen because they represent the main species found in the diet of grey seals around Britain (Prime and Hammond 1990; Hammond et al. 1994*a*, 1994*b*). Prey were obtained either commercially, through collaboration with the Fisheries Research Services (FRS) Marine Laboratory at Aberdeen, or were caught locally from the Gatty Marine Laboratory's RV *Orion* (which has a Scottish Office licence to catch and land undersize specimens). We fed otoliths and beaks in situ in whole prey rather than using a carrier species. This protocol better mimics feeding in the wild, and the method of otolith delivery is important because it affects digestion (Grellier and Hammond 2005). Because of the availability of prey at different times during the experimental period, different combinations of the 18 species were offered to each individual. In addition, the seals' individual preferences meant that they did not always eat every species they were offered.

Fish otolith and squid beak analysis

Uneaten remains (whole prey, fish heads or squid buccal masses, loose otoliths or beaks) were recovered on the first drain after the experimental meal was fed. Lengths of whole fish and weights of whole squid were measured directly. The length and width of loose otoliths and those removed from fish heads were also measured, and the length of the fish that they came from was inferred using the regressions shown in Table 3. The lower rostral length (LRL) of uneaten beaks was measured, and squid weight was inferred in the same way as for otoliths (Table 3). *F* and appropriate *t* tests were used to compare lengths of fish and weights of squid offered with those that were uneaten. This was important because a significant ($P < 0.05$) difference meant that the seal had consumed only part of the size range of prey offered, and therefore the size of the otoliths or beaks consumed could not be inferred and accurate digestion coefficients could not be calculated. If there was no significant difference between the size of the prey offered and those that remained uneaten, we assumed that the size distribution of fish offered was representative of those eaten. Trials with a significant difference were discarded.

Following the same protocol used for the analysis of scats collected in the wild, otoliths and beaks that had been eaten and recovered from each trial were identified to species, counted, and measured. Where they could not be identified with 100% certainty, they were excluded from further analysis. Broken otoliths and beaks were only counted and measured if the widest or longest part of the otolith or the LRL of the beak was complete. Otolith lengths (OL) and widths (OW) were measured to the nearest 0.01 mm using digital callipers (Mitutoyo) under a binocular microscope (PZO MST130). The callipers were zeroed between measurements and were

Table 3. Regressions used to infer prey size from otoliths and beaks that were not eaten.

Species	OL or LRL regression	r^2	*n*	OW regression	r^2	*n*	Reference
Short-spined sea scorpion	FL = 3.49OL − 1.37	0.90	196	FL = 7.23OW − 1.52	0.90	200	Leopold et al. 2001
Dragonet	FL = 8.41OL − 5.48	0.92	237	FL = 17.33OW − 5.19	0.92	249	Leopold et al. 2001
Atlantic cod	FL = 3.49OL − 6.64	0.88	268	FL = 7.84OW − 5.51	0.86	275	Leopold et al. 2001
Haddock	FL = 2.01OL + 3.71	0.43	237	FL = 5.65OW + 3.69	0.50	241	Grellier and Hammond 2005; Grellier 2006
Common dab	FL = 5.43OL − 3.49	0.88	261	FL = 8.88OW − 5.40	0.90	261	Leopold et al. 2001
European plaice	FL = 4.85OL − 2.07	0.76	405	FL = 8.15OW − 4.70	0.79	405	Leopold et al. 2001
Squid	$W = 17.33LRL^{2.21}$	0.77	193	—	—	—	The CIAC Beak Database (http://www.cephbase.utmb.edu)

Note: Units are as follows: fish length (FL; cm), otolith length (OL; mm), otolith width (OW; mm), squid weight (W; g) and lower rostral length (LRL; mm).

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frequently cleaned. Cephalopod beak LRLs were photographed using a microscope (Zeiss Axioskop 2 plus) and digital camera (Zeiss AxioCam) and measured to the nearest 0.01 µm using image-measurement software (AxioVision 3.1).

Recovery rates

The proportion of otoliths eaten that was recovered at the end of each feeding trial was calculated as the recovery rate. A value of 1 thus meant that all otoliths eaten were recovered; a value of 0 meant that no otoliths were recovered.

The theoretical variance of recovery rate was calculated as $p(1 - p)/n$, where p is the recovery rate and n is the number of otoliths that were eaten. Recovery rates and variances from each trial were combined to give single values for each seal for each prey species–size combination. Each trial was given equal weight. These values were then sequentially combined (giving equal weight to each seal) to give single values for each prey species–size combination, for each prey species, and finally, for each prey type (all *Trisopterus* spp. (Norway pout, *Trisopterus esmarkii*, poor cod), all large gadoids (Atlantic cod, *Gadus morhua*; haddock, *Melanogrammus aeglefinus*; European hake, *Merluccius merluccius*; whiting), flounder–plaice, all flatfish). Number correction factors were calculated as the inverse of the recovery rate (Bowen 2000).

Passage rates

Cumulative recovery rates calculated on a daily basis for each feeding trial were combined to give mean rates for each seal, each prey species–size combination, and each prey species. The prey species were then grouped according to their final recovery rate values. In addition, the percentage of the total number of otoliths and beaks recovered was calculated on a cumulative daily basis for large gadoids, *Trisopterus* spp., flatfish, sandeel (*Ammodytes marinus*), squid, and all fish species combined.

Species-specific digestion coefficients

All trials from which <10 otoliths were recovered ($n = 7$ for OL; $n = 2$ for OW) were excluded from further analyses. The small number of otoliths recovered in some of the remaining large gadoid trials was unavoidable because of the constraints of feeding large fish and maintaining a constant meal size. Digestion coefficients (mean otolith or beak size offered divided by mean otolith or beak size recovered) were calculated for each seal for each trial for both OL and OW (otoliths) and for LRL (beaks). The variance of each digestion coefficient was calculated using the delta method (Seber 1982; Grellier and Hammond 2005). The digestion coefficients and variances from each trial were combined in the same way as for recovery rates to give single values for each prey species and prey type.

Grade-specific digestion coefficients

Each recovered otolith was examined, and the amount by which it had been digested was classified based on its external morphological features (after Leopold et al. 2001; see Appendix A, Fig. A1). Pristine otoliths were classified as grade 1, moderately digested otoliths as grade 2, and considerably digested otoliths as grade 3. The amount by which beaks had been digested was not classified. Where ≥10 otoliths of each grade were recovered from a trial, grade-specific digestion coefficients and variances were calculated and combined in the same way as for species-specific digestion coefficients.

Results

A total of 13 845 otoliths and beaks of the 18 prey species were eaten by seven adult female grey seals during 86 feeding trials. 9650 (69.7%) of these otoliths and beaks were recovered from scats. There was no major loss of beads from the system over the course of the experiments; bead recovery rate was very high at 0.998 (2811/2818 beads were recovered). In 2004, we observed that on windy days, a small number of beads ($n = 4$) were caught in the wind while they were being scattered and actually fell outside the enclosure (i.e., they did not enter the system). We suggest that this is also the reason beads appeared to have been lost from the system in 2002 ($n = 5$) and 2003 ($n = 2$). Otoliths and beaks could not have been lost from the system in this way because they were deposited by seals at ground level or underwater, rather than being thrown through the air.

Recovery rates

We show the inter-individual variability and, where a prey species had been offered to the same individual multiple times, intra-individual variability in recovery rates for different prey species (Fig. 1). Recovery rates from trials with large gadoid prey varied from 0.605 to 1, with a high mean recovery rate of large gadoid otoliths of 0.935 (Table 1). Recovery rate was >0.9 in 75% of large gadoid trials, including 12 trials where the recovery rate was 1. Recovery rates from trials with *Trisopterus* spp. prey varied from 0.514 to 0.993; mean *Trisopterus* spp. otolith recovery rate was also high at 0.920 (Table 1). Recovery rate was >0.9 in 88% of *Trisopterus* spp. trials. By comparison, flatfish otolith recovery rates were lower (mean flatfish otolith recovery rate 0.806; only 43% of trials had recovery rates >0.9) and more variable (recovery rates from trials with flatfish varied from 0.318 to 1) (Table 1; Fig. 1). Atlantic herring (*Clupea harengus*) otolith recovery rates were highly variable, ranging from 0 to 0.909 for the 12 trials carried out (mean Atlantic herring otolith recovery rate 0.349). Mean sandeel otolith recovery rate was low (0.350; range 0.112 to 0.506). Squid beak recovery rates were consistently high (>0.9 in all trials). Similar numbers of upper ($n = 353$) and lower ($n = 354$) beaks were recovered (Table 1); mean recovery rate was 0.940 for upper beaks and 0.942 for lower beaks. Combined standard errors of the recovery rate estimates ranged from 0 (recovery rate = 1.0) to 0.079 (Table 1).

Recovery rate was positively correlated with mean undigested otolith size (Fig. 2; correlation coefficient 0.555 for OL and 0.603 for OW) and varied between different size ranges of the same prey. As expected, mean recovery rates of the large haddock, European hake, and whiting otoliths were greater than those for small otoliths (Table 1). This pattern was reversed for Atlantic cod (although only one small Atlantic cod trial was carried out).

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Fig. 1. Recovery rates from each trial (showing intra- and inter-individual variability). Each symbol represents a different seal. (*a*) Large gadoids, (*b*) flatfish, (*c*) other species.

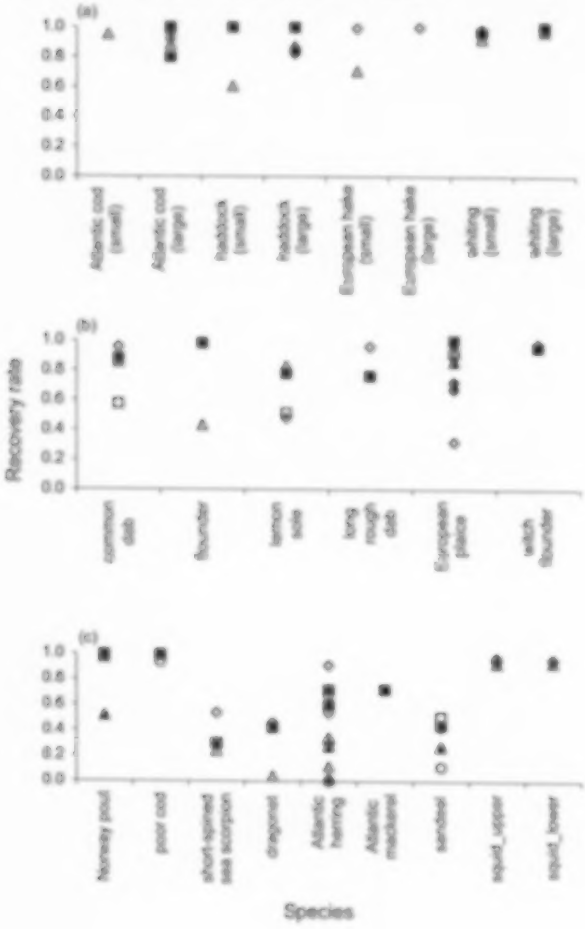
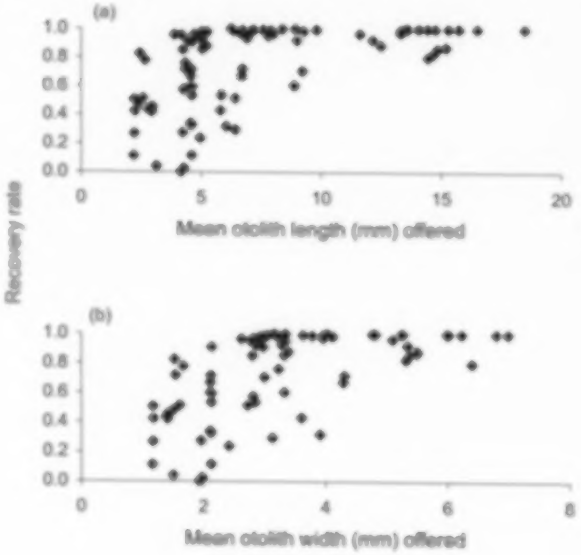


Fig. 2. Recovery rate plotted against mean undigested otolith size for all trials; larger otoliths were less likely to be completely digested than smaller otoliths. (*a*) Otolith length, (*b*) otolith width.



Passage rates

Greater than 95% of all otoliths and 70% of all beaks that were recovered had been passed by the third day after feeding (i.e., within 64 h; Table 4). All otoliths that were going to be recovered had been passed by the sixth day (136 h), all beaks by the seventh day (160 h). Passage rates varied among prey types; for example, 27% of sandeel otoliths were passed the day after feeding (within 16 h) compared with ≤7% for the other fish prey types. Upper squid beaks were passed more quickly than lower beaks.

Passage rates varied among prey species although it was hard to discern any characteristic differences between species that had high or medium final recovery rates (Fig. 3). For species that had low final recovery rates, the majority of otoliths were recovered on the second day after feeding (i.e., within 40 h). No consistent differences in passage rate were found between large and small otoliths of the same species.

Species-specific digestion coefficients

The inter- and intra-individual variability in digestion coefficients derived from each trial is shown (Fig. 4). Digestion coefficients calculated using OL were different from those calculated using OW, but the pattern of values was similar. Variability in both OL and OW digestion coefficients was higher in some species (e.g., large Atlantic cod and haddock) than in others (e.g., whiting). Digestion coefficients varied among prey species (Table 5). Mean large gadoid and sandeel digestion coefficients were relatively large (1.54 and 1.56 for OL, 1.52 and 1.65 for OW, respectively) compared with those for flatfish (1.25 for OL, 1.31 for OW) and *Trisopterus* spp.(1.21 for OL, 1.18 for OW). Standard errors of the estimates ranged from 0.011 to 0.120.

Where multiple size classes of a prey species were fed, mean digestion coefficients were greater for the larger size class in all four cases for OL and three out of four cases for OW (Table 5) (i.e., larger otoliths were proportionally more digested than smaller otoliths). This trend was consistent across species, but the relationship was weak (Fig. 5; correlation coefficient 0.497 for OL and 0.378 for OW).

Squid beaks were relatively undigested (mean LRL digestion coefficient 1.02; Table 5), and there was little variability in coefficients derived from different trials (Fig. 4).

Grade-specific digestion coefficients

Of the otoliths recovered from feeding trials, 0.8% were assigned a grade 1 (pristine), 8.5% a grade 2 (moderately digested), and 90.7% a grade 3 (considerably digested). Because relatively few grade 1 and grade 2 otoliths were recovered from feeding trials, it was only possible to calculate three grade-specific digestion coefficients for one prey

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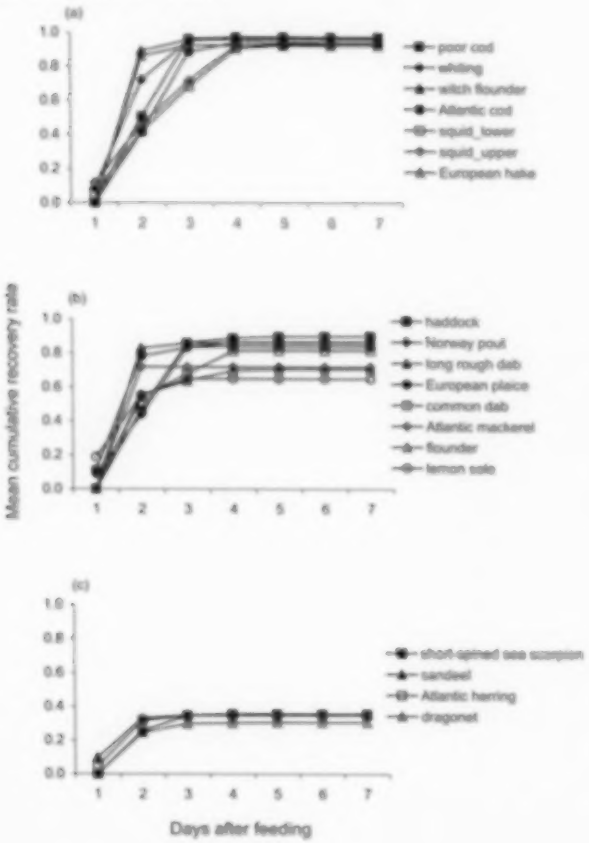
Table 4. The percentage of the total number of otoliths and beaks recovered calculated per day.

Species	Day 1 (16 h)	Day 2 (40 h)	Day 3 (64 h)	Day 4 (88 h)	Day 5 (112 h)	Day 6 (136 h)	Day 7 (160 h)
Trisopterans	5.02	51.42	97.60	99.86	99.98	100.00	100.00
Sandeel	27.19	94.46	99.79	100.00	100.00	100.00	100.00
Large gadoids	2.16	65.83	96.88	99.56	99.91	100.00	100.00
Flatfish	7.05	85.27	95.24	99.76	100.00	100.00	100.00
All fish species	5.94	77.40	96.96	99.72	99.98	100.00	100.00
Squid_upper	12.40	49.16	76.44	98.23	99.41	100.00	100.00
Squid_lower	5.47	44.67	72.63	96.34	98.55	99.64	100.00

Note: The figures shown in parentheses in the first row of the table are the approximate number of hours after feeding that the otoliths or beaks were recovered.

Fig. 3. Species-specific passage rates. (*a*) High final recovery rate (>0.9). (*b*) Medium final recovery rate (0.6–0.9). (*c*) Low final recovery rate (<0.4).



species (Atlantic herring). Two grade-specific digestion coefficients were calculated for six prey species, and a single grade-specific coefficient was calculated for five species (six species when grade-specific digestion coefficients were calculated using OW). For three species, measurements from grade 1 and grade 2 otoliths were pooled, and a "non-grade 3" digestion coefficient was calculated in addition to the grade 3 digestion coefficient (Table 6).

As with species-specific digestion coefficients, grade-specific digestion coefficients calculated using OL were different from those calculated using OW (Table 6). Combined standard errors of the estimates were small so that in (almost) all species, grade 2 estimates plus 2 standard errors were smaller than grade 3 estimates minus 2 standard errors.

Where more than one size class of a prey species was fed, mean digestion coefficients were greater for the larger size class for the same grade in all four cases for OL and in three out of four cases for OW (Table 6) (i.e., larger otoliths were proportionally more digested than smaller otoliths).

Discussion

Experimental protocol

We fed otoliths in situ in whole prey rather than using a carrier species to present the otoliths or heads of the prey items of interest (see Tollit et al. 1997; Marcus et al. 1998; Berg et al. 2002). This protocol better mimics feeding in the wild, and the method of otolith delivery in experiments is important because removal of otoliths from the skull case affects their digestion by seals; while fish size can be accurately estimated using digestion coefficients derived from in situ experiments, application of digestion coefficients derived from carrier experiments results in an overestimation of fish size (Grellier and Hammond 2005).

The seven seals used in this study were captured in the wild and kept in captivity for the duration of the feeding experiment, after which time they were released back into the wild. While this made carrying out the feeding experiments challenging, the digestion correction factor estimates are likely to be more applicable to otoliths and beaks recovered from scats collected in the wild than those derived from the majority of captive feeding studies with phocids, which have used either animals that were born in captivity or those that were long-term captives (e.g., Prime and Hammond 1987; Cottrell et al. 1996; Tollit et al. 1997).

The fact that the seals used in this study were captured as adults in the wild may have contributed to their general willingness to accept a varied diet. We found that some individuals accepted a more varied diet than others, and certain individuals had very definite prey preferences. This suggests that populations of seals are more likely to be a collection of specialists rather than each individual being a generalist, or at least that a population comprises specialists as well as generalists. Further evidence to corroborate this suggestion

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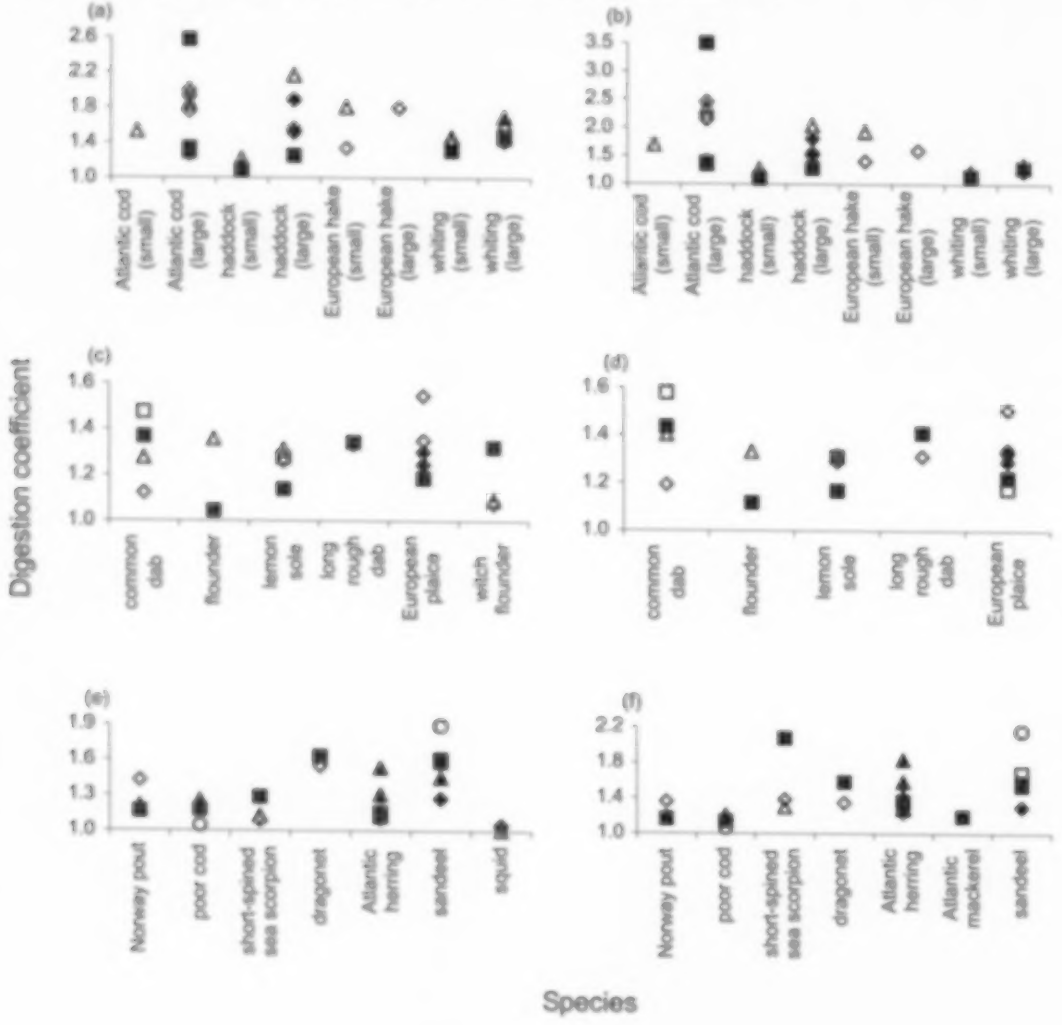
Fig. 4. Digestion coefficients from each trial (showing intra- and inter-individual variability). Each symbol represents a different seal. For Atlantic mackerel (*Scomber scombrus*), only otolith width could be used because the length of all of the recovered otoliths was incomplete. For witch flounder (*Glyptocephalus cynoglossus*), only otolith length could be used because no suitable regression for predicting otolith width from fish length was available. (*a*) Large gadoid otolith length digestion coefficients, (*b*) large gadoid otolith width digestion coefficients, (*c*) flatfish otolith length digestion coefficients, (*d*) flatfish otolith width digestion coefficients, (*e*) other species otolith length and lower rostral length digestion coefficients, (*f*) other species otolith width digestion coefficients.



comes from a study on the movements and foraging areas of grey seals caught in the North Sea and tracked using Argos satellite relay dataloggers (McConnell et al. 1999). The duration, extent, and regularity of foraging trips varied considerably, both from seal to seal and through time. Although there was variability in individual behaviour, the fact that specific movement patterns of individuals were observed repeatedly suggests that some grey seals are specialists, targeting sources of preferred prey at certain times of year.

We fed single-species meals to estimate digestion coefficients, recovery rates, and passage rates for the major prey of British grey seals. Although otoliths of single species (in particular sandeel) are commonly recovered from scats collected in the wild, otoliths of several prey species may also be recovered from a single scat (Grellier 2006). Casper et al. (2006) suggested that their low recovery rates (from a mixed-prey feeding experiment using *Arctocephalus* spp. seals) may be a feature of mixed prey diets. However, because they did not also feed single-species meals no comparison was made, and therefore, this statement cannot be qualified. Notwithstanding this, it would be interesting to investigate whether otolith digestion is affected by meal composition (for grey seals and the most commonly found combinations of their North Sea prey). However, this issue

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Table 5. Species-specific digestion coefficients (dc) calculated for grey seals (*Halichoerus grypus*).

Species	dc (SE)	No. of seals	No. of trials	No. of otoliths or lower beaks recovered
Otolith length or lower rostral length				
Norway pout	1.25 (0.013)	4	4	1413
Poor cod	1.17 (0.016)	4	4	862
All trisopterans	1.21 (0.014)	5	8	2275
Short-spined sea scorpion	1.12 (0.042)	1	1	18
Dragonet	1.59 (0.044)	2	2	153
Atlantic herring	1.18 (0.029)	4	9	288
Sandeel	1.56 (0.033)	5	5	1123
Atlantic cod_large	1.63 (0.080)	3	7	108
Atlantic cod_small	1.53 (0.061)	1	1	33
Atlantic cod	1.58 (0.071)	3	8	141
Haddock_large	1.66 (0.064)	4	5	157
Haddock_small	1.14 (0.017)	3	3	408
Haddock	1.40 (0.047)	4	8	565
European hake_large	1.79 (0.087)	1	1	24
European hake_small	1.57 (0.038)	2	2	283
European hake	1.68 (0.068)	2	3	307
Whiting_large	1.58 (0.041)	3	3	174
Whiting_small	1.40 (0.016)	3	3	961
Whiting	1.49 (0.031)	4	6	1135
All large gadoids	1.54 (0.057)	5	25	2148
Common dab	1.31 (0.042)	4	4	326
Flounder	1.20 (0.055)	2	2	54
Lemon sole	1.25 (0.042)	4	4	288
Long rough dab	1.34 (0.024)	2	2	517
European plaice	1.26 (0.056)	5	7	277
Witch flounder	1.16 (0.027)	3	3	229
Flounder–plaice	1.23 (0.056)	6	9	331
All flatfish	1.25 (0.043)	6	22	1691
Squid	1.02 (0.023)	2	3	354
Otolith width				
Norway pout	1.22 (0.011)	4	4	1454
Poor cod	1.14 (0.018)	4	4	895
All trisopterans	1.18 (0.015)	5	8	2349
Short-spined sea scorpion	1.58 (0.111)	3	3	57
Dragonet	1.47 (0.039)	2	2	189
Atlantic herring	1.40 (0.047)	4	10	446
Atlantic mackerel	1.19 (0.031)	1	1	23
Sandeel	1.65 (0.030)	5	5	1151
Atlantic cod_large	1.92 (0.120)	3	8	136
Atlantic cod_small	1.69 (0.069)	1	1	39
Atlantic cod	1.81 (0.098)	3	9	175
Haddock_large	1.63 (0.056)	4	5	203
Haddock_small	1.17 (0.016)	3	3	495
Haddock	1.40 (0.041)	4	8	698
European hake_large	1.59 (0.066)	1	1	42
European hake_small	1.67 (0.037)	2	2	370
European hake	1.63 (0.053)	2	3	412
Whiting_large	1.30 (0.023)	4	4	319
Whiting_small	1.19 (0.011)	3	3	1143
Whiting	1.24 (0.018)	4	7	1462
All large gadoids	1.52 (0.060)	5	27	2747
Common dab	1.40 (0.039)	4	4	375
Flounder	1.22 (0.037)	2	2	72
Lemon sole	1.27 (0.043)	4	4	306
Long rough dab	1.36 (0.026)	2	2	550

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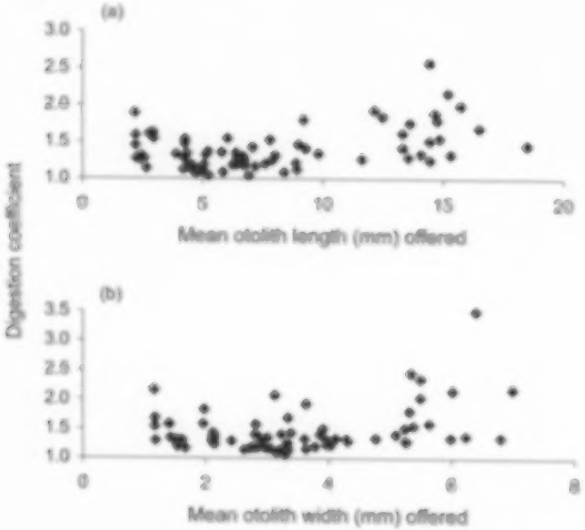
Table 5 (*concluded*).

Species	dc (SE)	No. of seals	No. of trials	No. of otoliths or lower beaks recovered
European plaice	1.27 (0.042)	5	7	329
Flounder–plaice	1.25 (0.039)	6	9	401
All flatfish	1.31 (0.038)	6	19	1632

Fig. 5. Digestion coefficient plotted against mean undigested otolith size for all trials; larger otoliths were proportionally more digested than smaller otoliths. (*a*) Otolith length, (*b*) otolith width.



is complex because one scat does not represent one meal; parts of a meal, or multiple meals, or both, may be represented in a single scat.

Recovery rates

Analyses of scat samples to infer marine mammal diets are subject to bias if species-specific differences in number correction factors are not taken into account (Bowen 2000). Our results showed that number correction factors for grey seal prey were all greater than 1 and ranged up to 3. As expected, recovery rates (reciprocal of number correction factor) were greater for species with large otoliths (i.e., large otoliths were less likely to be completely digested) (Tollit et al. 1997, 2003; Grellier and Hammond 2005). These species-specific differences in number correction factors are particularly important because the proportions that each prey species represent in the diet will be altered by different amounts, and the impact of number correction factors will vary with the mixture of prey species consumed. When the last comprehensive assessment of British grey seal diet was carried out in 1985, sandeels and large gadoids (particularly Atlantic cod) dominated the diet in all areas and most months of the year (Prime and Hammond 1990; Hammond et al. 1994*a*, 1994*b*). The mean number correction factors of 2.86 (sandeel) and 1.06 (Atlantic cod) estimated here mean that the contribution of sandeels was likely underestimated and vice versa for Atlantic cod. These data are currently being reanalysed. Bowen (2000) noted that the use of number correction factors tends to decrease the importance of gadoids and other species with large, robust otoliths in the diets of pinnipeds.

Although recovery rates for large fish were greater than those for small fish of the same species, it is unclear how size-specific number correction factors might be applied to otoliths recovered from scats collected in the wild (because their size will have been altered according to the amount of digestion they have undergone). By conducting experiments with prey of a size range representative of grey seal diet in the wild and by calculating a single species-specific recovery rate (by combining recovery rates from trials by individual and then by prey species–size combination, prey species, and finally prey type), we have incorporated variability in the final recovery rate estimate and minimized any potential bias.

Number correction factors can only be applied if at least one otolith or beak of a species has been recovered. Because recovery of structures is probabilistic, the failure to find a single structure does not necessarily mean that no items of this prey species were consumed. This may have implications for determining how to group scats collected over a range of sites and times. The smaller the number of scats grouped for analysis, the higher the probability of not recovering a single otolith. In practice, this problem will generally arise for species that are rare, and therefore relatively unimportant, in the diet. However, it may be especially important in studies where prey species of particular interest have these characteristics, for example Salmonidae.

Passage rates

We found some species-specific differences in passage rates, but the large majority of otoliths and beaks were passed after relatively short periods (2 or 3 days). This suggests that grey seal diet estimated using scat analysis is likely to be representative of actual diet in the North Sea, where mean trip length is short (2.3 days; McConnell et al. 1999) and at-sea distribution is primarily coastal (a 35 km zone around the coasts was found to include 60% of the population's total at-sea usage; Matthiopoulos et al. 2004). Differences in the distribution and abundance of prey may still affect how representative scat analysis is. However, modelling of passage rate data in combination with data on prey distribution and seal foraging and haulout behaviour has shown no bias for grey seals around Britain (Smout 2006). This issue is important and should be considered when assessing the diet of other species in other areas.

Species-specific digestion coefficients

Large otoliths had greater digestion coefficients than small otoliths; this trend was consistent across species. Therefore, the size of prey with large otoliths (i.e., larger prey) would be most underestimated if digestion coefficients were not

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Table 6. Grade-specific digestion coefficients (dc) calculated for grey seals (*Halichoerus grypus*).

Species	Grade	dc (SE)	No. of seals	No. of trials	No. of otoliths recovered
Otolith length					
Norway pout	3	1.25 (0.013)	4	4	1399
Poor cod	3	1.17 (0.016)	4	4	858
All trisopterans	3	1.21 (0.014)	5	8	2257
Dragonet	2	1.22 (0.048)	2	2	28
	3	1.70 (0.041)	2	2	125
Atlantic herring	1	1.04 (0.021)	1	2	25
	2	1.11 (0.021)	3	7	144
	3	1.32 (0.035)	3	3	36
Sandeel	2	1.25 (0.041)	2	2	43
	3	1.58 (0.034)	5	5	1070
Atlantic cod_large	3	1.60 (0.063)	3	6	98
Atlantic cod_small	3	1.53 (0.061)	1	1	33
Atlantic cod	3	1.56 (0.062)	3	7	131
Haddock_large	3	1.66 (0.064)	4	5	157
Haddock_small	3	1.14 (0.017)	3	3	406
Haddock	3	1.40 (0.047)	4	8	563
European hake_large	3	1.84 (0.080)	1	1	23
European hake_small	3	1.62 (0.037)	2	2	270
European hake	3	1.73 (0.062)	2	3	293
Whiting_large	3	1.59 (0.041)	3	3	166
Whiting_small	1+2	1.08 (0.028)	1	1	10
	3	1.40 (0.016)	3	3	942
Whiting	1+2	1.08 (0.028)	1	1	10
	3	1.50 (0.031)	4	6	1108
All large gadoids	1+2	1.08 (0.028)	1	1	10
	3	1.55 (0.052)	5	24	2095
Common dab	2	1.10 (0.020)	2	2	127
	3	1.38 (0.045)	4	4	193
Flounder	2	1.01 (0.024)	1	1	27
	3	1.26 (0.073)	2	2	25
Lemon sole	1+2	1.11 (0.042)	4	4	144
	3	1.46 (0.055)	4	4	144
Long rough dab	2	1.05 (0.022)	1	1	32
	3	1.36 (0.024)	2	2	478
European plaice	1+2	1.10 (0.039)	2	2	25
	3	1.29 (0.061)	5	7	242
Witch flounder	2	1.00 (0.015)	1	1	10
	3	1.18 (0.028)	3	3	208
Flounder–plaice	3	1.28 (0.068)	6	9	267
All flatfish	1+2	1.10 (0.041)	4	6	169
	3	1.32 (0.051)	6	22	1290
Otolith width					
Norway pout	3	1.22 (0.011)	4	4	1439
Poor cod	3	1.14 (0.018)	4	4	891
All trisopterans	3	1.18 (0.015)	5	8	2330
Short-spined sea scorpion	3	1.76 (0.109)	2	2	33
Dragonet	2	1.11 (0.041)	2	2	28
	3	1.54 (0.040)	2	2	161
Atlantic herring	1	1.08 (0.020)	1	2	25
	2	1.23 (0.028)	3	8	177
	3	1.63 (0.055)	4	9	191
Atlantic mackerel	3	1.22 (0.045)	1	1	14
Sandeel	2	1.22 (0.046)	2	2	43
	3	1.68 (0.030)	5	5	1096

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Table 6 (*concluded*).

Species	Grade	dc (SE)	No. of seals	No. of trials	No. of otoliths recovered
Atlantic cod_large	3	1.92 (0.120)	3	8	134
Atlantic cod_small	3	1.69 (0.069)	1	1	39
Atlantic cod	3	1.80 (0.098)	3	9	173
Haddock_large	3	1.63 (0.056)	4	5	203
Haddock_small	3	1.17 (0.016)	3	3	492
Haddock	3	1.40 (0.041)	4	8	695
European hake_large	3	1.62 (0.065)	1	1	41
European hake_small	2	1.09 (0.028)	1	1	10
	3	1.72 (0.036)	2	2	356
European hake	2	1.09 (0.028)	1	1	10
	3	1.67 (0.053)	2	3	397
Whiting_large	3	1.30 (0.022)	4	4	311
Whiting_small	1+2	1.03 (0.017)	1	1	13
	3	1.19 (0.011)	3	3	1118
Whiting	1+2	1.03 (0.017)	1	1	13
	3	1.24 (0.018)	4	7	1429
All large gadoids	1+2	1.03 (0.017)	1	1	13
	3	1.53 (0.060)	5	27	2694
Common dab	2	1.16 (0.022)	2	2	128
	3	1.48 (0.042)	4	4	241
Flounder	2	1.06 (0.020)	1	1	27
	3	1.28 (0.053)	2	2	43
Lemon sole	1+2	1.12 (0.045)	4	4	144
	3	1.47 (0.060)	4	4	162
Long rough dab	2	1.05 (0.021)	1	1	32
	3	1.39 (0.026)	2	2	511
European plaice	1+2	1.11 (0.042)	2	2	25
	3	1.29 (0.043)	5	7	294
Flounder–plaice	3	1.29 (0.048)	6	9	337
All flatfish	1+2	1.12 (0.043)	4	6	169
	3	1.38 (0.046)	6	19	1251

applied to otoliths recovered from scats. By calculating a species-specific digestion coefficient based on otoliths recovered from meals of fish of a size range representative of what seals eat in the wild, we have incorporated this variability in the final digestion coefficient estimates and minimized any potential bias. An alternative approach would be to feed meals of fish of many (small) size classes of each species and model undigested otolith size as a function of digested size, thereby allowing the correction to vary with digested size. This approach could also be taken with the recovery rate data if experiments were conducted in this way.

We calculated digestion coefficients based on both otolith length and width for all fish prey, except Atlantic mackerel, *Scomber scombrus* (the lengths of all recovered Atlantic mackerel otoliths were incomplete) and witch flounder, *Glyptocephalus cynoglossus* (no suitable regression for predicting otolith width from fish length was available). When deciding whether to apply an OL or OW digestion coefficient to otoliths recovered from scats collected in the wild, the balance between sample size and variability in the digestion coefficient estimates must be considered. In some cases, the decision will be simple. For example, the tips of whiting otoliths are often missing. Therefore, digestion coefficients based on OW are likely to be more useful for this species so that a larger number of otoliths can be used to estimate the size of prey consumed. The other piece of information relevant to this decision is how good is the relationship between otolith length or width and fish size. In addition, standard errors of the OW digestion coefficients for whiting are smaller than those for the OL coefficients.

Grade-specific digestion coefficients

Intraspecific variation is likely to be caused by a number of factors, such as meal size, frequency and species composition (Marcus et al. 1998), and level of activity of the seal (Tollit et al. 2003). By applying a suitable digestion coefficient based on the external morphological features of the otolith, intraspecific variation can be taken into account whatever its cause.

Application of grade-specific digestion coefficients to account for intraspecific variation in size reduction caused by digestion appears to be the most promising approach to reconstructing prey size (Tollit et al. 2004). While labour intensive, the need to apply grade-specific digestion coefficients can be evaluated by estimating the proportion of time an inappropriate digestion coefficient would be used if only a single digestion coefficient were applied. This is particu-

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larly important because average digestion rates appear to be artificially high in captive seals (Tollit et al. 1997; this study). The percentage of otoliths of each grade recovered from scat samples collected from around the North Sea during 2002 of 0.2% grade 1, 34% grade 2, and 65.8% grade 3 (Grellier 2006) was in contrast with the proportion of each grade recovered from feeding experiments (this study). Because average digestion rates appear to be artificially high in captive seals, application of species-specific digestion coefficients derived from captive feeding experiments will introduce bias. This is because the undigested size of otoliths recovered from scats collected in the wild will be overestimated a substantial proportion of the time. Because the proportion of otoliths assigned to each grade varies among species, use of species-specific digestion coefficients will affect prey differently. Therefore, overall diet composition estimates of wild seals may be biased unless (*i*) the proportion of otoliths of each grade are similar for each prey species for wild and captive seals, (*ii*) digestion coefficients for a species are similar among grades, or (*iii*) grade-specific digestion coefficients are applied.

In some cases, for example when reanalysing previous data where otoliths were not graded, the only option is to use species-specific digestion coefficients. We investigated the sensitivity of estimates of diet composition to using species-specific rather than grade-specific digestion coefficients for the first quarter of 2002 in Orkney (North Sea; Grellier 2006). The relative amount of sandeels in the diet increased by around 4% and the relative amount of Atlantic cod decreased by around 5% when species-specific rather than grade-specific digestion coefficients were used. The amounts of other gadoid prey also decreased except for saithe (i.e., pollock, *Pollachius virens* (not to be confused with European pollock, *Pollachius pollachius*)) and European ling (*Molva molva*), which increased. The relative contribution of flatfish also generally decreased. Overall then, only small biases resulted from using species-specific rather than grade-specific digestion coefficients.

The high proportion of grade 3 otoliths recovered from scats collected during these feeding trials (and in the North Sea in 2002; Grellier 2006) suggests that it would be useful to refine the wear class system and split this grade into two or even three subgrades; this would be an improvement to the present system and would further reduce bias.

Comparison with other studies

Previous studies on the rate of otolith digestion by grey seals are limited to those by Prime and Hammond (1987), Hammond and Prime (1990), and Marcus et al. (1998). Methodological differences mean that a fair comparison of recovery rate, digestion coefficient, and passage rate values can be made only for Atlantic herring, because the method of otolith delivery affects digestion (Grellier and Hammond 2005). (All otoliths in this study were fed in situ in whole fish, while a carrier was used to present otoliths to seals in the other studies, with the exception of Atlantic herring otoliths in the study by Marcus et al. (1998).) The Atlantic herring otolith recovery rate in this study (0.349) was greater than that derived by Marcus et al. (1998) (0.244), while the passage rates and (OL) digestion coefficients were similar in both studies. Both systems yielded consistently high bead recovery rates, and the length of Atlantic herring fed was similar; therefore, the most likely explanation for the observed difference in recovery rate concerns the small number of otoliths fed in each meal by Marcus et al. (1998). When just 10 otoliths are fed (as they were in half the trials in Marcus et al. (1998)), the percentage difference one otolith makes to the recovery rate is greater than the difference made when more otoliths are fed. Factors such as the difference in age (and level of activity) of the seals used in experiments (all seals used in this study were adults; all those used by Marcus et al. (1998) were pups) may also have contributed (e.g., Warner 1981 cited in Casper et al. 2006).

Importance of work

The interaction between grey seals and fisheries is a contentious issue (e.g., Moore 2003). It is crucial to provide unbiased estimates of diet with associated measures of uncertainty, particularly given that management actions may be taken based on such estimates. These digestion correction factors are the most systematically obtained and most comprehensive for any species of pinniped and will allow accurate and precise estimation of the number and size of fish represented by otoliths recovered from grey seal scat samples collected in the wild. They will be used to update estimates of grey seal diet composition and consumption of commercially important species (Grellier 2006), which in turn will provide government with the best available data on which to base policy and management decisions.

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Appendix A

Appendix appears on the following page.

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Fig. A1. Images of pristine (grade 1; upper image), moderately digested (grade 2; lower left image), and considerably digested (grade 3; lower right image) otoliths used to classify otoliths to create relative correction factors. No wear classes were listed for witch flounder or European hake; for these species, we used wear classes shown for species with similar otoliths (long rough dab (*Hippoglossoides platessoides*) and whiting, respectively). (Images were taken and edited by Cindy van Damme and have been reproduced with permission from NIOZ (Royal Netherlands Institute for Marine Research), Alterra, and ETI (Expert Centre for Taxonomic Identification). Also refer to Leopold et al. (2001).)

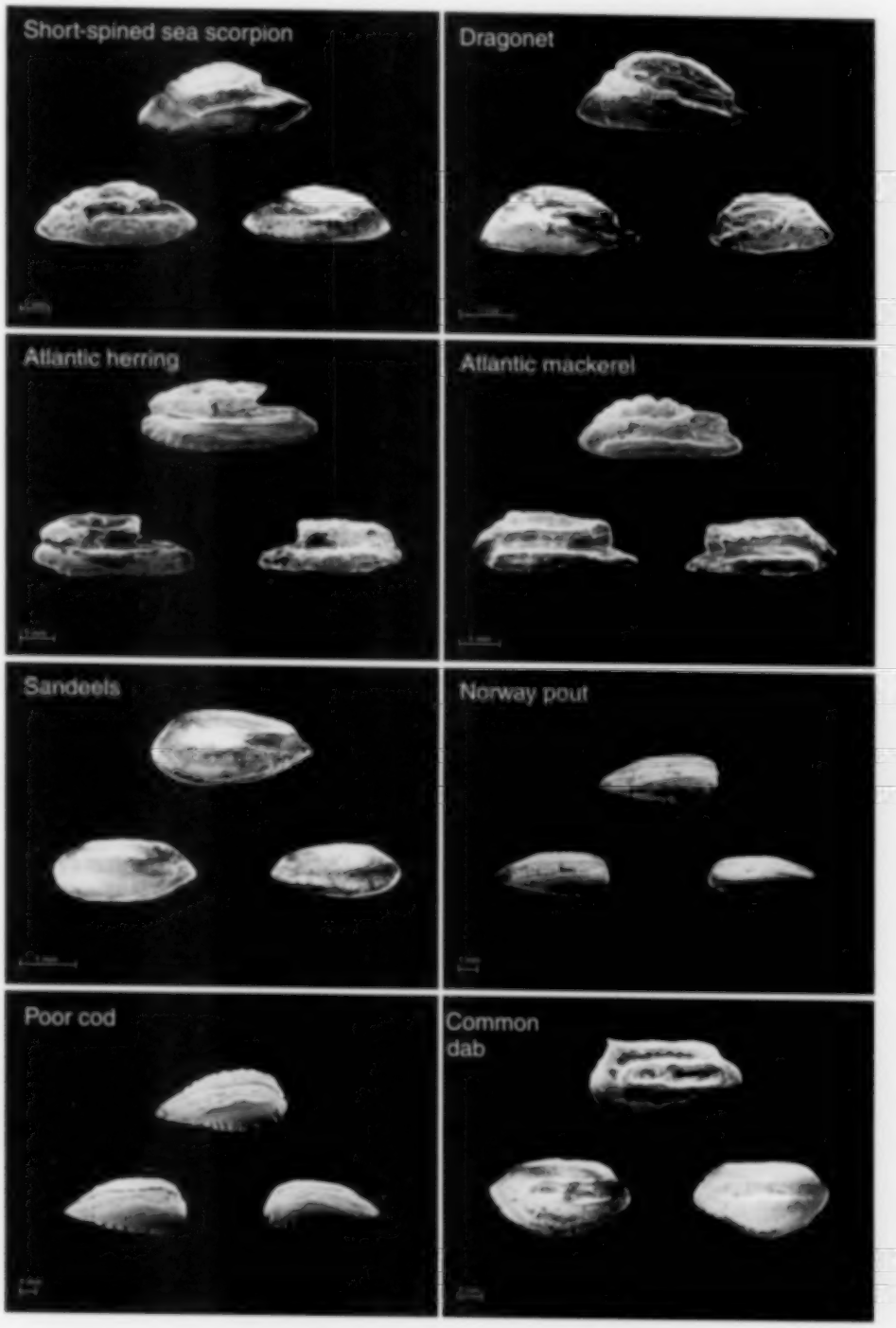

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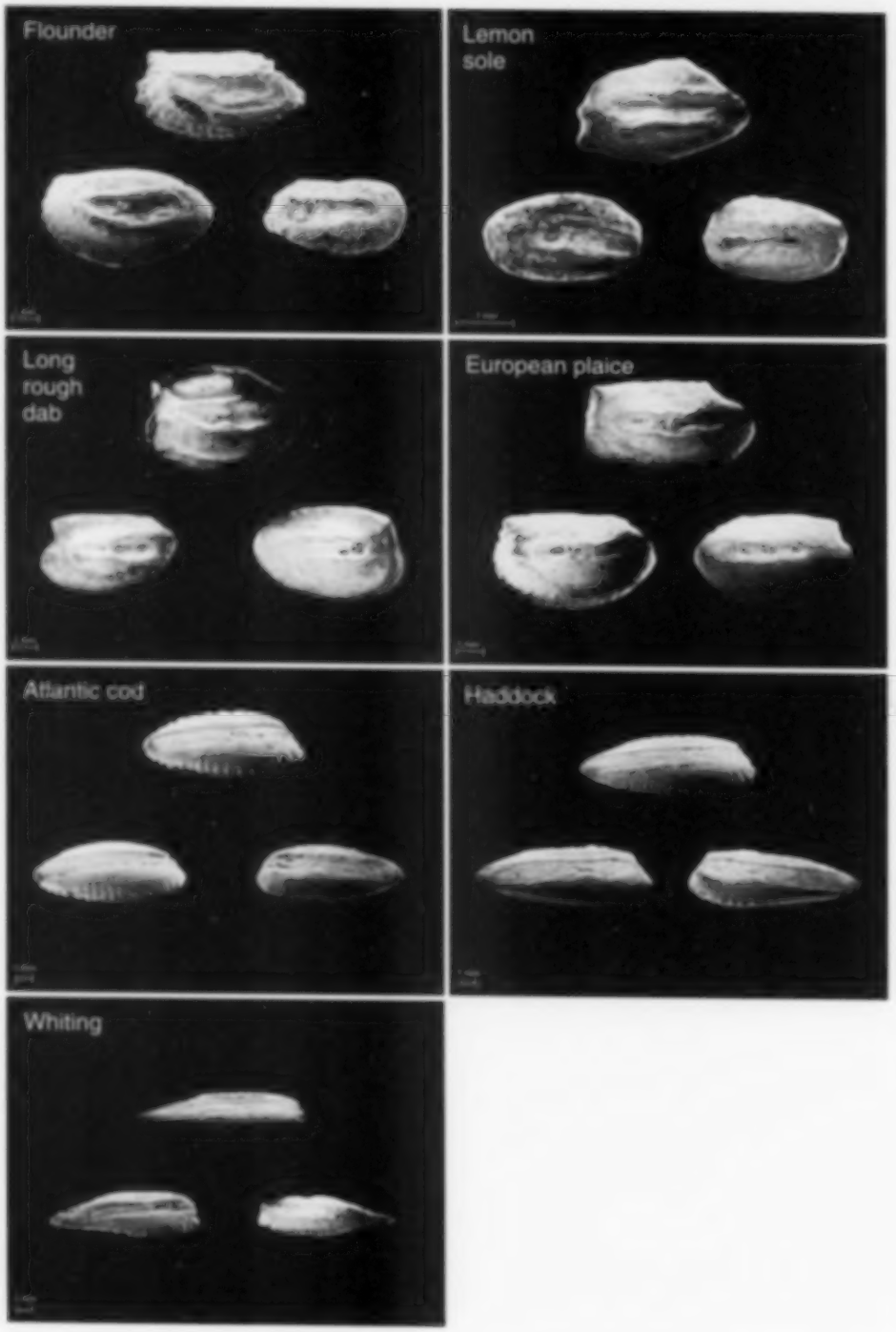
Fig. A1 *(concluded).*



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Benthic grazing and functional compensation in stressed and recovered lakes

Kristy L. Hogsden and Rolf D. Vinebrooke

Abstract: During ecosystem recovery, grazing pressure is expected to increase as larger herbivores become reestablished. Alternatively, grazing pressure may remain unchanged during recovery as large consumers replace and functionally compensate for more abundant populations of smaller, tolerant herbivores. We tested these hypotheses by conducting a 90-day experiment in which three size categories of benthic consumers were excluded from producers in three chemically stressed and three recovered lakes. Our findings showed that consumers did not significantly affect producer biomass in either type of lake. However, exposure to larger and more abundant grazers did induce a physiognomic shift towards less edible producers in the recovered lakes. In comparison, recovered lakes contained significantly greater producer biomass and diversity. Comparison of the observed subtle effects of consumers and pronounced negative impact of ecosystem stress on benthic producers suggest that they can compensate for natural disturbances (e.g., grazing), but not for the other multiple stressors associated with anthropogenic acidification of the Killarney lakes.

Résumé : Durant la restauration des écosystèmes, on s'attend à ce que la pression de broutement augmente, à cause du retour des grands herbivores. D'un autre côté, la pression de broutement peut rester inchangée durant la restauration, parce que les consommateurs de grande taille remplacent les populations plus abondantes d'herbivores tolérants plus petits et compensent fonctionnellement pour eux. Nous avons testé ces hypothèses lors d'une expérience de 90 jours durant laquelle trois tailles de consommateurs benthiques ont été séparées des producteurs dans trois lacs en stress chimique et trois lacs restaurés. Nos résultats montrent que les consommateurs n'affectent pas significativement la biomasse des producteurs dans les deux types de lacs. Cependant, une exposition à des brouteurs plus abondants et de plus grande taille induit un changement physionomique qui favorise les producteurs moins comestibles dans les lacs restaurés. En comparaison, les lacs restaurés possèdent une biomasse et une diversité de producteurs significativement plus importantes. La comparaison des effets subtils des consommateurs que nous observons et l'important impact négatif du stress de l'écosystème sur les producteurs benthiques indiquent que ces derniers peuvent compenser pour les perturbations naturelles (par ex., le broutement), mais pas pour les autres multiples facteurs de stress reliés à l'acidification anthropique des lacs de Killarney.

[Traduit par la Rédaction]

Introduction

Ecological processes are expected to become more efficient as species diversity and size structure increase in ecosystems during recovery from anthropogenic stressors (Odum 1985). More diverse consumer assemblages can better exploit food resources (Naeem and Li 1998; Norberg 2000; Duffy 2002), while larger consumers often exert stronger predation pressure than do small-bodied, stress-tolerant species (Brooks and Dodson 1965; Cattaneo and Kalff 1986; Diehl 1992). For example, reestablishment of a more diverse assemblage of larger herbivores has been hypothesized to increase grazing pressure in recovering acidified lakes (Fairchild and Sherman 1992; Vinebrooke et al. 2003*a*; Hogsden and Vinebrooke 2005).

Alternatively, ecological processes may remain unchanged during ecosystem recovery owing to compensatory species dynamics (i.e., functional compensation; Frost et al. 1995; Klug et al. 2000; Fischer et al. 2001). For instance, sparse populations of large, sensitive consumers replace higher densities of smaller, acid-tolerant species in recovering acidified lakes (Hogsden and Vinebrooke 2005), thereby possibly maintaining net secondary production and grazing pressure. Further, producers may also functionally compensate or even overcompensate for shifts in grazing pressure and other environmental changes in acidified lakes (Vinebrooke 1996; Klug et al. 2000; Vinebrooke et al. 2003*b*).

Functional compensation involving shifts in the body sizes of benthic consumers has not been addressed empirically in anthropogenically stressed lakes. Previous experiments that used only coarse-meshed (250 µm) exclosures showed that consumers affected producers in circumneutral lakes, but not in acidified lakes where large-bodied grazers were in low abundance (Vinebrooke 1996; Graham and Vinebrooke 1998). Therefore, we conducted an experiment in which three size categories of benthic consumers were excluded from producers to determine their relative impacts in stressed and recovered

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Table 1. Physical characteristics and acidification histories for six lakes included in the experiment (mean ± standard error (SE)).

Lake	Lake surface area (ha)	Depth (m)		Acidification history (pH)*		
		Mean	Max.	1971–1973	1992	2003
Stressed						
Acid	19.6	10.9	29	—	5.1	5.1
Norway	63.3	15.1	33.6	4.7	4.5	5.4
Ruth Roy	54.5	4.3	18	4.5	4.2	5.8
Mean	45.8	10.1	26.9	4.6	4.6	5.4
SE	13.4	3.1	4.6	0.1	0.3	0.2
Recovered						
A.Y. Jackson	6.5	7	9.8	5	5.6	6.2
Carlyle	156.7	5.7	14.6	5	5.1	6.3
Little Sheguiandah	4.5	1.7	2.7	5	6.3	6.9
Mean	55.9	4.8	9	5	5.7	6.5
SE	50.5	1.6	3.5	0	0.3	0.2

*Acidification history data (1971–1973 and 1992) were taken from Vinebrooke and Graham (1997); 2003 data is from Hogsden and Vinebrooke (2005). Note that data was not available for Acid Lake for 1971–1973.

lakes. We hypothesized that a compensatory size shift from numerous, small, tolerant consumers towards fewer, larger, sensitive consumers maintains grazing pressure as lakes recover from acidification. We also expected that a shift in the size of grazer would alter feeding selectivity, thereby affecting producer diversity. Further, we predicted that relatively diverse producer assemblages would show greater functional compensation than species-poor, higher consumer groups, such as herbivores (e.g., Tilman 1999; Klug et al. 2000; Vinebrooke et al. 2003*b*).

Materials and methods

Study area

The experiment was conducted from May to August 2003 in six lakes located in Killarney Provincial Park (46°3′N, 81°21′W), southwest of Sudbury, Ontario, Canada. Reduction of anthropogenic emissions of sulphur dioxide has resulted in chemical improvements in many of these acid-stressed lakes (Snucins et al. 2001; Findlay 2003; Keller et al. 2003). Lakes were selected based on their historical and current acidities, which are useful abiotic determinants of ecosystem recovery from anthropogenic acidification (Vinebrooke and Graham 1997; Keller et al. 2003). The "stressed" lake group consisted of three acidic waterbodies (Acid, Norway, and Ruth-Roy) situated on orthoquartzite bedrock, while the "recovered" group included three chemically recovered (i.e., returned to pre-acidification pH), circumneutral lakes (A.Y. Jackson, Carlyle, and Little Sheguiandah) located in lowland areas with limestone, granite, or sandstone bedrock (Snucins et al. 2001). All lakes in the stressed and recovered lake groups became acidified below pH 5 in the early 1970s but currently differ in chemical recovery status (Table 1; Vinebrooke and Graham 1997; Vinebrooke et al. 2003*a*; Hogsden and Vinebrooke 2005).

Lake water chemistry

Chemical analyses were performed on water samples collected monthly during summer 2003 from the site of the experiment in each lake. Each study area was located on a south-facing bedrock outcropping that provided a shallow-sloping littoral habitat and a 1 m submerged rock ledge that ran parallel to the shore. Duplicate samples were analyzed at the Limnology Laboratory at the University of Alberta (Edmonton, Alberta, Canada). In the lab, pH was measured using a Fischer Accumet model 925 meter and compared with values recorded during summer sampling. Conductivity was determined using a radiometer (Copenhagen model CDM83). Samples for total nitrogen (TN) samples were predigested with alkaline potassium persulfate according to Ameel et al. (1993) and determined by flow injection analysis on a Technicon Autoanalyzer™. Total dissolved phosphorus (TDP) was measured using standard colorimetric procedures following digestion with potassium persulfate (Menzel and Corwin 1965). Dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC) were both analyzed using a Shimadzu TOC-5000A analyzer.

Consumer communities

Zoobenthos were collected monthly from the shallow (~1 m depth) littoral area using a D-frame dip net (500 µm mesh size). Three time-standardized sweeps were performed with the net along a 10 m transect adjacent to the experimental site and parallel to the shoreline. Samples were pooled and stored in coolers until sorting was performed later in the day. Predators were immediately separated from the samples during sorting. A dissecting microscope was used at 40× and 100× magnifications to sort and identify all invertebrates to the family level. Many invertebrates were collected as early instars and were difficult to identify to genus or species; therefore, family level was used for all statistical analyses. Body size was measured at the widest part of the body (usually head or abdomen) and was divided into four size classes (<0.5, 0.5–2.5, 2.5–10, and >10 mm) based on accessibility into the different experimental exclosures (see below). Consumers were also classified according to published dietary descriptions and functional feeding groups (Merritt and Cummins 1984; Thorp and Covich 2001). We use the term consumer to refer collectively to all functional feeding groups (i.e., consumer = predators + grazers + omnivores).

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For statistical analyses, the grazer fraction is considered separately when compared with other consumers (i.e., predators plus omnivores). After drying at 60 °C for 96 h, samples were weighed. Dry mass for snails and crayfish were shell-free.

Experimental design and setup

A two-factor, split-plot design with repeated measures was used to determine the effects of chemical recovery and size structure of benthic consumer communities on autotrophic biomass and species composition. The lake-type treatment (plot) consisted of two levels (stressed pH ~5, recovered pH ~7) that were replicated three times for a total of six lakes. The lakes served as independent experimental units because of the absence of connecting streams. Four consumer treatment levels (subplot) were created using open, coarse (10 mm), intermediate (2.5 mm), and fine (0.5 mm) mesh exclosures (40.6 cm long × 27.9 cm wide × 15.2 cm deep) that were closed on the bottom. Consumer treatment levels were not replicated within any of the lakes. A single partial cage for each level of the consumer treatment was also deployed in each lake to account for the physical presence of the exclosure material (i.e., cage effect). Partial cages were constructed using the same design and materials of the complete exclosures; however, they lacked vertical walls. Each exclosure contained a matrix of 12 unglazed ceramic tiles (56.25 cm^2 each), which simulated the predominant type of algal habitat (quartzite–granitic bedrock) that exists along the shallow shorelines of the study lakes. All ceramic tiles were acid washed (10% HCl) for 48 h prior to deployment. In each lake, cages were randomly deployed along a submerged rock ledge and secured using bricks in early May 2003.

Sampling and enumeration of producers

Four tiles from each exclosure were retrieved randomly and processed monthly for a total of three sampling events. Producers were removed from the four tiles with a hard-bristled toothbrush, rinsed with filtered lake water, and pooled together. The harvested material was transferred from the tray to a 500 mL graduated cylinder and was filled to 200 mL with filtered lake water. The top of the cylinder was covered and inverted several times to mix thoroughly and was subsampled in the following sequence: 50 mL was filtered and concentrated on Whatman GF/C glass microfiber filter papers and frozen for chlorophyll *a* (Chl *a*) analysis, and 100 mL was preserved with Lugol's solution for taxonomic analysis. Producer biomass was quantified using fluorimetry and biovolume estimates (Wetzel and Likens 2000). For Chl *a* determinations, samples were extracted in 95% ethanol for 24 h. Tubes were centrifuged at 5 °C and 4400 $r \cdot min^{-1}$ for 10 min, and dilutions, if required, were made with 95% ethanol. Extracts were read on a Shimadzu model RF-1501 spectrofluorophotometer at 436 nm and 680 nm, using a 1 cm path-length quartz cell. For biovolume estimates, samples were well mixed and diluted depending on the density of algal and detrital material. After 1 h of settling into the base of an Utermöhl chamber, a minimum of 300 viable cells were counted under 400× or 1000× magnification on an inverted microscope. Cell viability was based on chloroplast integrity for eukaryotic cells and pigmentation for cyano-

Table 2. List of periphytic producers used in canonical correspondence analysis (CCA).

No.	Taxon	Algal group	Physiognomic group
1	*Anabaena*	C	LU
2	*Aphanocapsa*	C	LU
3	*Chroococcus*	C	LU
4	*Lyngbya*	C	LU
5	*Merismopedia*	C	LU
6	*Oscillatoria*	C	LU
7	*Phormidium*	C	LC
8	*Rhabderma*	C	LU
9	*Dinobryon*	CHR	LU
10	*Actinotaenium*	DE	LU
11	*Bambusina*	DE	LC
12	*Closterium*	DE	LU
13	*Cosmarium*	DE	LU
14	*Cylindrocystis*	DE	LU
15	*Desmidium*	DE	LC
16	*Euastrum*	DE	LU
17	*Gonatozygon*	DE	LU
18	*Netrium*	DE	LU
19	*Penium*	DE	LU
20	*Pleurotaenium*	DE	LU
21	*Staurastrum*	DE	LU
22	*Xanthidium*	DE	LU
23	*Achnanthes*	DI	FU
24	*Actinella*	DI	FU
25	*Cymbella*	DI	FU
26	*Eunotia*	DI	LU
27	*Fragilaria*	DI	LC
28	*Frustulia*	DI	LU
29	*Gomphonema*	DI	FC
30	*Melosira*	DI	LC
31	*Navicula*	DI	LU
32	*Nedium*	DI	LU
33	*Pinnularia*	DI	LU
34	*Semiorbis*	DI	LU
35	*Stenopterobia*	DI	LU
36	*Surirella*	DI	LU
37	*Synedra*	DI	FU
38	*Tabellaria*	DI	LU
39	*Bulbochaete*	FG	FC
40	*Klebsormidium*	FG	LC
41	*Mougeotia*	FG	LC
42	*Oedogonium*	FG	FC
43	*Spirogyra*	FG	LC
44	*Stigeoclonium*	FG	FC
45	*Zygogonium*	FG	LC
46	*Chlamydomonas*	G	LU
47	*Pediastrum*	G	LU
48	*Scenedesmus*	G	LU
49	*Stichococcus*	G	LU

Note: Taxon identification numbers correspond to those used in CCA plots. Taxa are identified as cyanobacteria (C), chrysophytes (CHR), desmids (DE), diatoms (DI), filamentous green algae (FG), and green algae (G) and are grouped in to loose understory (LU), loose canopy (LC), firm understory (FU), or firm canopy (FC) categories based on their growth form and inferred attachment potential.

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Table 3. Water chemistry for stressed and recovered lakes (mean ± standard error (SE)) over the course of the 90-day experiment based on sampling in littoral area.

			Lake type		Time		Time × lake type	
Variable	Stressed	Recovered	*F*	*P*	*F*	*P*	*F*	*P*
pH	5.43±0.18	6.42±0.16	12.24	0.02	1.29	0.32	0.13	0.94
DOC ($mg \cdot L^{-1}$)	2.02±0.15	3.92±0.25	14.21	0.02	0.66	0.59	3.09	0.07
DIC ($mg \cdot L^{-1}$)	0.88±0.27	1.20±0.21	0.51	0.52	1.64	0.23	0.21	0.89
TDP ($\mu g \cdot L^{-1}$)	1.93±0.35	2.89±0.66	167.68	0.04	4.87	0.11	1.12	0.47
TN ($\mu g \cdot L^{-1}$)	248.42±10.23	307.16±12.15	11.96	0.03	1.50	2.64	0.35	0.80
Conductivity ($\mu S \cdot cm^{-1}$)	25.19±2.46	26.73±2.24	0.03	0.88	0.74	0.55	0.20	0.89

Note: DOC, dissolved organic carbon; DIC, dissolved inorganic carbon; TDP, total dissolved phosphorus; and TN, total nitrogen.

Table 4. Level of accessibility to experimental exclosures for consumer families in stressed and recovered lakes.

	Stressed				Recovered			
Family	Fine	Intermediate	Coarse	Open	Fine	Intermediate	Coarse	Open
Hyallidae		×				×		
Gammaridae							×	
Ranidae								×
Dytiscidae (adult)	×						×	
Dytiscidae (larvae)			×					
Gyrinidae			×					
Cyprinidae								×
Cambaridae			×				×	
Chironomidae	×				×			
Ephemerellidae						×		
Heptageniidae						×		
Viviparidae							×	
Corixidae			×					
Notonectidae			×					
Hydrachnida		×				×		
Asellidae		×				×		
Corydalidae			×				×	
Sialidae						×		
Libellulidae			×				×	
Gomphidae			×				×	
Coenagriondae		×					×	
Limnephilidae			×				×	

Note: Taxa missing from table indicate families that were absent from each lake type.

bacteria. Cell counts were converted to biovolumes by approximating cell volume using geometric formulae (Hillebrand et al. 1999; Sun and Liu 2003). Biovolume was estimated on a per-cell basis for solitary and filamentous cells. A minimum of 20 cells per taxon was used to determine the mean dimensions used in geometric formulae, where sufficient cell numbers were available. Biovolume estimates were converted to biomass assuming the specific gravity equaled one and mean values derived from duplicate or triplicate enumerations. Comparison of benthic producers that had been sampled from acid-washed tiles and freshly collected rocks during a 2003 survey of these lakes indicated that producer communities on tiles contained greater average species richness (49 taxa vs. 30 taxa) (Hogsden and Vinebrooke 2005). *Homoeothrix*, a relatively rare and slow-growing cyanobacterium, was the only genus found on the rocks that was absent from the tiles. Therefore, representative sampling of the benthic producer community was achieved using tiles in each lake. All algal samples were identified to genus according to Prescott (1982) and Wehr and Sheath (2003). Identification to this level of taxonomic resolution generalized species within a genus and was a limitation of our study design. Duplicate counts of each sample were done, and if species richness (i.e., number of species) varied by more than 20%, a triplicate sample was counted.

To assess ecological responses by producers to grazing and lake type, taxa were grouped into four physiognomic categories based on their individual morphologies and abilities to attach to surfaces (Table 2; Peterson 1996; Steinman 1996; Graham and Vinebrooke 1998). The physiognomy of benthic producers serves as a highly reliable predictor of their responses to several environmental factors, including grazing (Steinman 1996), resource availability (Burkholder 1996), and physical disturbance (Peterson 1996). Taxa that possessed a stalked attachment structure or specialized basal holdfast were termed firmly attached. In contrast, other taxa

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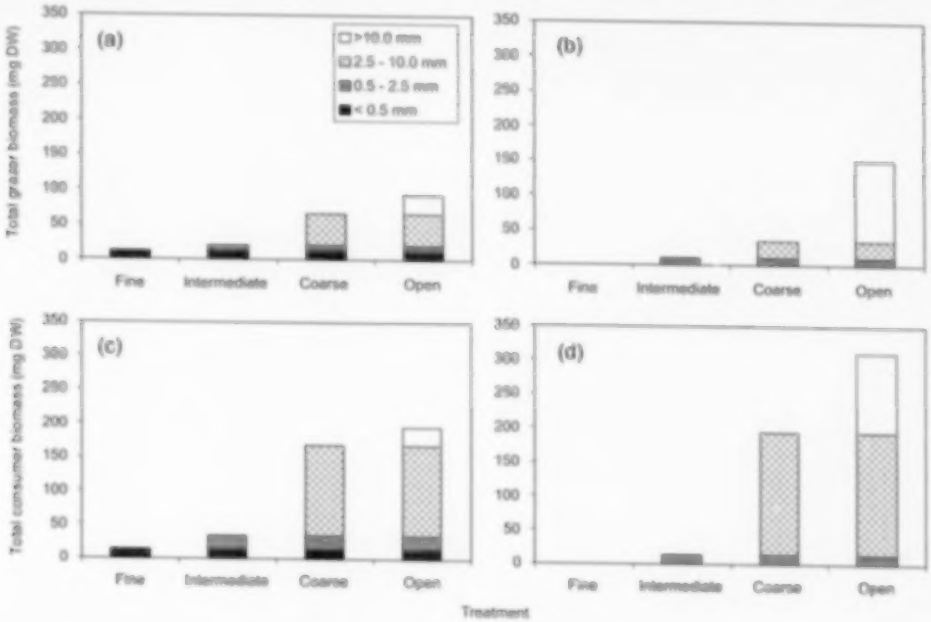
Fig. 1. Biomass of each size fraction of (*a*) grazers in stressed lakes, (*b*) grazers in recovered lakes, (*c*) consumers in stressed lakes, and (*d*) consumers in recovered lakes that can access each exclosure treatment level (data presented are mean biomass (DW, dry weight) over entire experiment).



that lacked tightly adhering structures were defined as weakly attached. Firmly and loosely attached groups were subdivided into canopy and understory categories based on the stature of each taxon. Canopy taxa included filamentous and long-stalked growth forms, while understory taxa were prostrate or short-stalked.

Although the physiognomic approach can help simplify interpretations of ecological responses by producers, several caveats pertain to its use. For example, mucilaginous excretions by taxa that lacked specific attachment structures may have enabled them to better adhere to the tiles. Secondly, heterotrichous taxa (e.g., *Stigeoclonium*) can produce both erect canopy and prostrate understory filaments. However, this alga was classified as a canopy producer because its erect filaments were more abundant than basal cells. Thirdly, certain genera were represented by species that differ in their growth forms (e.g., stalked and nonstalked *Achnanthes* spp). In these cases, the relative abundance of identifiable species and their associated growth form were used to classify each genus into one of the four physiognomic groups.

Statistical analyses

Repeated measures analysis of variance (RM-ANOVA) was used to evaluate differences in water chemistry, the effectiveness of experimental exclusion of consumers, and the effects of lake type and consumer size on producer biomass (Chl *a* and biomass) (Scheiner and Gurevitch 2001). Lake type was included as a between-plot factor, and consumer size was included as a within-plot factor. Statistical analysis was performed with SYSTAT Version 10 (SPSS Inc., Chicago, Illinois) using the general linear model procedure. If the assumption of sphericity was violated, we used conservative Greenhouse–Giesser-corrected significance levels ($P < 0.05$). Repeated-measures multivariate analysis of variance (RM-MANOVA) was used to determine differences in the physiognomic and taxonomic composition of the producers between stressed and recovered lakes and grazer treatment levels. All data were $\log_{10}$-transformed to improve normality and homogeneity of variance, which were verified using Shapiro–Wilks and Levene's tests, respectively.

A specific set of statistical results were required to support our hypothesized effect of a compensatory size-shift by consumers maintaining grazing pressure across acidified and recovered lakes. First, we would have to detect a significant time-dependent interaction between lake type and consumer size treatments. If so, then multiple-comparison testing would be required to determine the nature of the interaction. Specifically, pronounced grazing by small, acid-tolerant consumers would be demonstrated if producers remained significantly more abundant in the fine-meshed exclosures compared with both open controls and coarser-meshed cages placed in the acidified lakes. In comparison, a significantly negative difference of a similar magnitude between coarser-meshed exclosures and open controls in the recovered lakes would indicate a compensatory size shift towards larger grazers.

Canonical correspondence analysis (CCA) was conducted using CANOCO version 4.51 (2003, Biometris-Plant Research International, Wageningen, the Netherlands) to examine the effect of water chemistry variables and grazer biomass on producer assemblages. All algal, grazer, and water chemistry data, with the exception of pH, used in the ordination were $\log_{10}$-transformed to stabilize variances (Morin and Cattaneo 1992) and to reduce the influence of large-celled taxa (e.g., filamentous green algae (FGA)), which would have dominated the ordination (ter Braak 1986). To remove ecologically irrelevant and insignificant or redundant environmental

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variables, data were screened prior to the final CCA using forward selection. Monte Carlo permutation testing (499 iterations) was used to test for the statistical significance of the final ordination. Algal taxa that did not occur in at least three samples were excluded from ordination analysis.

Results

Water chemistry

The water chemistry differed substantially between the two lake types (Table 3). Stressed and recovered lakes differed by approximately a full pH unit. Recovered lakes also contained significantly higher concentrations of TDP, TN, and photoprotective DOC (RM-ANOVA, lake type, $P < 0.05$). Conductivity and DIC levels were not significantly different across all six lakes.

Consumer communities

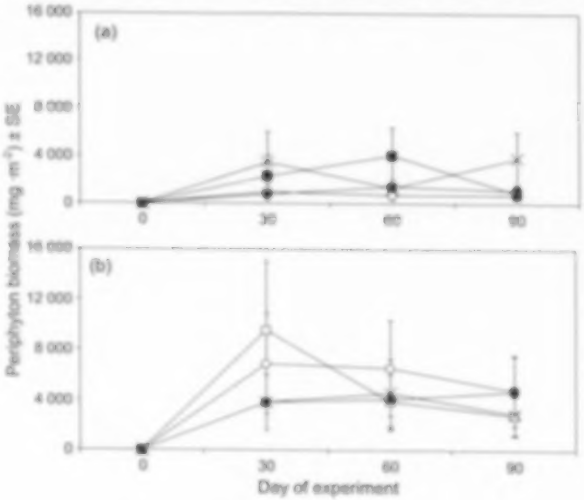
The fine-meshed exclosures potentially excluded most consumers, except chironomids and hydrachnids, from producers in both stressed and recovered lakes (Table 4). Small dytiscids could also enter the fine-meshed exclosures, but only in the stressed lakes. Intermediate-meshed exclosures could exclude ephemerillids and heptagenids that were only present in the recovered lakes. Similarly, coarse-meshed exclosures could prevent larger consumers (amphipods, viviparids, gammarids) accessing the tiles in the recovered lakes. The size of some consumers differed between stressed and recovered lakes and affected the treatment exclosure that could be accessed. For example, small coenagrionids entered the intermediate-meshed exclosures in the stressed lakes, while larger coenagrionids were excluded in the recovered lakes (Table 4).

Use of exclosures significantly reduced potential exposure of producers to all consumers (RM-ANOVA, consumer effect, $F_{[3,16]} = 7.28$, $P = 0.003$), but not all grazers (RM-ANOVA, consumer effect, $F_{[3,16]} = 5.74$, $P = 0.39$) during the entire experiment (Fig. 1). Multiple pairwise comparisons (Tukey's test) revealed significant differences in consumer biomass between each treatment level ($P = 0.005$), with biomass increasing as exclosure size increased. However, potential exposure did not differ significantly between acidified or recovered lakes for all total consumers (RM-ANOVA, lake type – consumer–time interaction, $F_{[6,32]} = 1.6$, $P = 0.17$) or grazers ($F_{[6,32]} = 0.7$, $P = 0.65$). There was an apparent shift from smaller grazers (e.g., chironomids) in stressed lakes to fewer but larger taxa (e.g., mayflies, tadpoles, minnows) in recovered lakes (Figs. 1*c*, 1*d*); however, it was not statistically significant (ANOVA, $P > 0.05$).

Lake-type and consumer-size effects on producer biomass

Exclusion of different size categories of consumers did not significantly affect total producer biomass in either stressed or recovered lakes (RM-ANOVA, lake type – consumer–time interaction, $F_{[6,24]} = 0.3$, $P = 0.95$; Fig. 2). In particular, total biomass did not differ significantly ($t = 0.76$, df = 7, $P = 0.47$) between fine-meshed exclosures and open controls by day 90 of the experiment. Chlorophyll-inferred producer biomass did not differ significantly between the partial-cage

Fig. 2. Benthic producer biomass in each exclosure in (*a*) stressed lakes ($n = 3$) and (*b*) recovered lakes ($n = 3$) over the 90-day experiment. Open circles, open exclosures; open squares, coarse exclosures; solid circles, intermediate exclosures; ×, fine exclosures.



treatments and open controls (RM-ANOVA, lake type – consumer–time interaction, $F_{[6,20]} = 0.5$, $P = 0.81$).

Acidified lake conditions significantly suppressed the total abundance of benthic producers based on differences in Chl *a* (RM-ANOVA, lake-type effect, $F_{[1,22]} = 31.4$, $P < 0.001$) and algal biomass ($F_{[1,24]} = 38.4$, $P < 0.001$) between the stressed and recovered lakes (Fig. 3). At the end of the experiment, both metrics revealed producer biomass had increased in the recovered lakes and remained relatively unchanged in the stressed lakes (Fig. 3). Total Chl *a* and producer biomass were positively correlated across all treatments ($R^2 = 0.25$, $P < 0.001$). Total producer biomass on experimental tiles was considerably less than recorded biomass on surveyed rocks during the same time period in both stressed and recovered lakes (Hogsden and Vinebrooke 2005).

Lake-type and consumer-size effects on producer diversity

The physiognomic structure of benthic producers did not differ significantly between stressed and recovered lakes or among the three exclosure types (Fig. 4; RM-MANOVA, lake type – consumer–time interaction, $F_{[33,132]} = 0.6$, $P = 0.94$). Loosely attached canopy taxa represented the most abundant growth form across all treatments.

Acidified lake conditions significantly altered producer diversity based on changes in the abundance of major algal groups (Fig. 5; RM-MANOVA, lake type – time interaction, $F_{[14,224]} = 7.0$, $P < 0.001$). Specifically, significantly more desmids (RM-ANOVA, lake-type effect, $F_{[1,22]} = 21.21$, $P < 0.001$), diatoms ($F_{[1,22]} = 53.95$, $P < 0.001$), FGA ($F_{[1,22]} = 13.61$, $P = 0.001$), and other green algae ($F_{[1,22]} = 9.50$, $P = 0.05$), but not cyanobacteria ($F_{[1,22]} = 1.02$, $P = 0.32$) occurred in recovered lakes. FGA was the most abundant algal group in the recovered lakes, representing over 90% of the total producer biomass by day 90. Diatoms were the second most abundant group in the recovered lakes, accounting for 7% of the final total producer biomass. In comparison,

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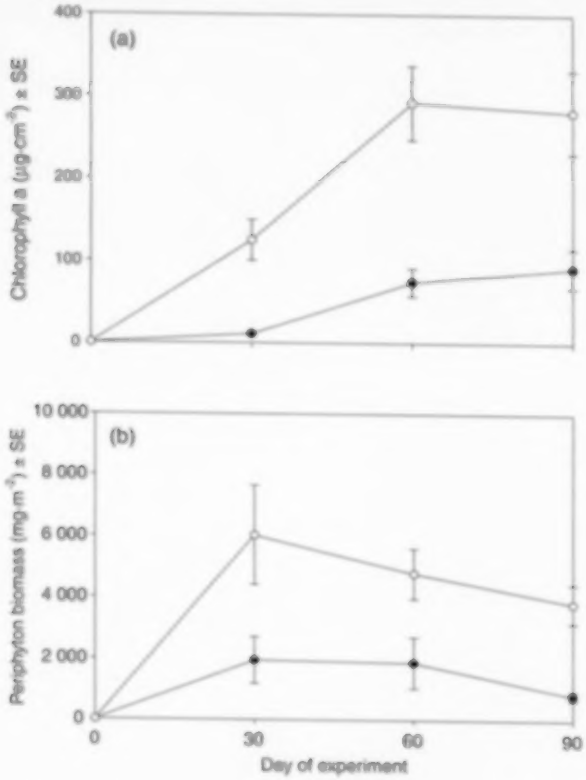

Fig. 3. (*a*) Chlorophyll *a* and (*b*) producer biomass in stressed lakes (solid circles) and recovered lakes (open circles) over the 90-day experiment.

FGA were less abundant by a full order of magnitude in the stressed lakes in which they still represented over 80% of the total producer biomass by day 90. Consumers did not significantly affect the abundance of major algal groups (RM-MANOVA, consumer–time interaction, $F_{[42,224]} = 0.4$, $P = 0.98$). Other green algae included all chlorophytes that did not belong to the Zygnematales or Oedogoniales. A summary of RM-ANOVA and RM-MANOVA analyses is provided in Table 5.

Chemical differences between the stressed and recovered lakes significantly altered producer diversity at the genus level based on CCA (Fig. 6). A total of 62 genera from six major taxonomic groups (cyanobacteria, chrysophytes, diatoms, desmids, FGA, and other green algae) were detected in the experiment. Chrysophytes made up less than 1% of total biomass and were excluded from further analysis. CCA was performed on 49 genera that accounted for 99% of the total producer biomass across all exclosures (Table 2). Fourteen genera were excluded from analysis, as they occurred in fewer than three samples. The first CCA axis explained 18.7% of the total taxonomic variance (Fig. 6*a*). CCA axis 1 ($\lambda_1 = 0.30$) represented a significant gradient ($F = 13.79$, $P < 0.005$) that was best described by DOC ($r = -0.95$) and pH ($r = -0.79$). The first axis contrasted genera (*Euastrum* (No. 16), *Pleurotaenium* (No. 20), and *Pinnularia* (No. 33)) that were common in recovered lakes, from filamentous green algae (*Stigeoclonium* (No. 44)) and desmids (*Actinotaenium* (No. 10) and *Cylindrocystis* (No. 14)) that were more abundant in the stressed lakes.

Consumers also significantly influenced producers at the genus level (Fig. 6*a*). Grazer biomass was retained as an independent, significant explanatory variable that was correlated ($r = 0.41$) with CCA axis 2 ($\lambda_2 = 0.10$). The second CCA axis explained 9% of the total taxonomic variance and was also related to DIC ($r = 0.78$) and conductivity ($r = 0.56$). CCA axis 2 separated grazer-resistant, firmly attached genera (*Gomphonema* (No. 29), *Achnanthes* (No. 23), and *Bulbochaete* (No. 39)) and *Xanthidium* (No. 22) in recovered lakes from loosely attached taxa, such as *Lyngbya* (No.4) and *Penium* (No. 19), in stressed lakes. Clear separation of recovered from stressed lakes was evident based on producer diversity (Fig. 6*b*).

Discussion

Our results provided partial support for the hypothesis that a shift from numerous small benthic consumers to fewer large grazers maintains secondary production and grazing pressure in recovering acidified lakes. Lack of a significant difference in total grazer biomass between the lake types was associated with large taxa (larval mayflies, tadpoles, minnows) being more abundant in the recovered lakes, while the stressed lakes contained greater densities of smaller grazers (chironomids). However, we did not detect a significant interactive effect of consumer size and lake type on producer biomass. Therefore, grazing pressure did not depend on different size categories of consumers in acidified and recovered lakes. Further, consumers did not exert a significant main effect on producer biomass across all lakes. Instead, the influence of grazing was only detected at the taxonomic (i.e., genus) level in recovered lakes based on ordination analysis. In contrast, other environmental stressors significantly suppressed producer biomass and altered taxonomic composition in the acidified lakes. These findings suggest that benthic producers functionally compensated for weak grazing pressure, but not for anthropogenic acidification. Below, we consider potential explanations for weak benthic grazing effects and differences in functional compensation by producers to environmental stressors and grazing in relatively unproductive lakes.

Benthic grazing may have been difficult to detect in these lakes because consumer–producer interactions can be weak in oligotrophic systems (Elser and Goldman 1991; Lamberti 1996; but see Rosemond et al. 1993). Low productivity combined with predation by benthivores (e.g., crayfish, odonates, minnows) can suppress smaller herbivorous invertebrates, thereby minimizing their effect on producers in stressed lakes without piscivorous fish (Appelberg et al. 1993). Further, the prevalence of omnivory (Gu et al. 1997) and flexibility in feeding strategy (Dangles 2002) likely weaken the reliance of many grazers on producers in stressed ecosystems. For example, stable isotopic analyses reveal that most benthic invertebrates are not obligate herbivores in unproductive lakes (Vander Zanden and Rasmussen 1999; Vinebrooke et al. 2001). Therefore, low population densities of omnivorous, benthic invertebrates likely preclude detection of grazing effects in oligotrophic systems.

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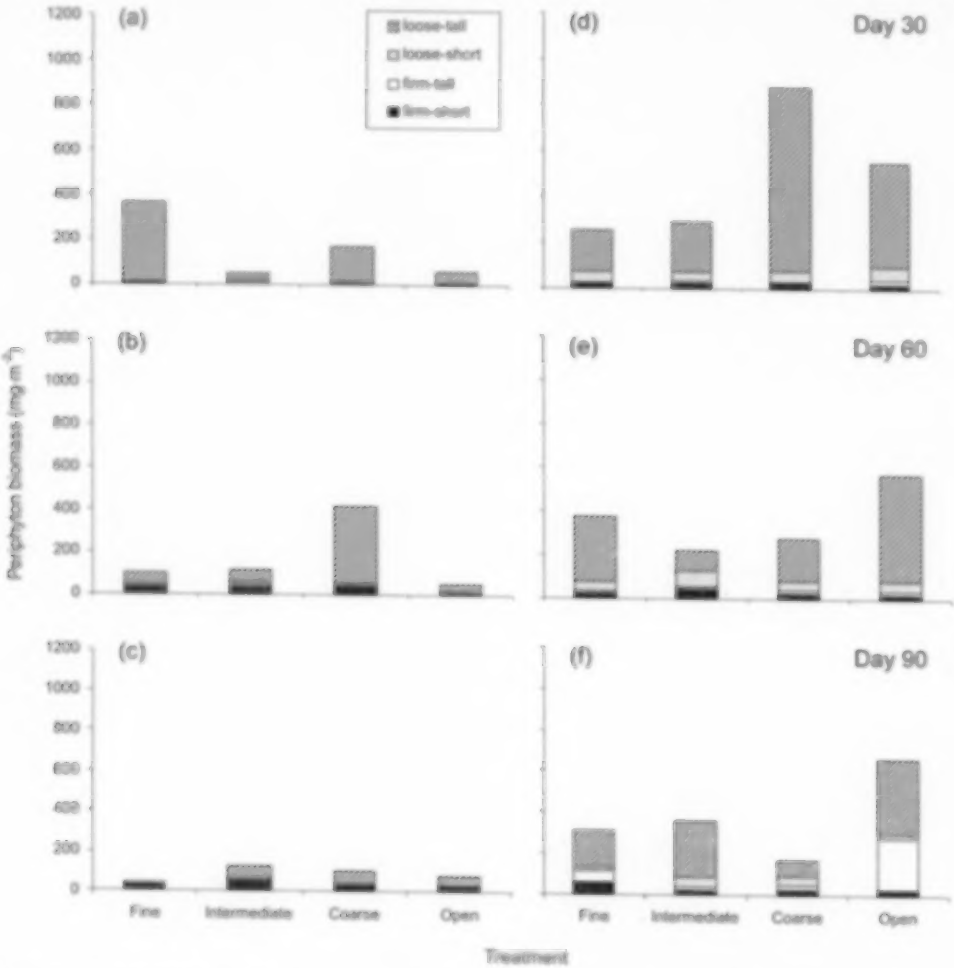
Fig. 4. Benthic producer biomass based on physiognomic groups in (*a*–*c*) stressed lakes and (*d*–*f*) recovered lakes for each exclosure treatment level.



High abundances of potentially edible producers in all lakes also suggested that ambient grazing pressure was weak during the study. Specifically, filamentous canopy producers were abundant in both stressed and recovered lakes despite their reported susceptibility to large-bodied grazers (Gelwick and Matthews 1992; Vinebrooke 1996; Graham and Vinebrooke 1998). Compensatory shifts involving edible (zygnematacean taxa) and relatively grazer-resistant filamentous algae (oedogoniacean taxa) may also have offset the effects of benthic grazers on producer biomass (e.g., Vinebrooke 1996; Graham and Vinebrooke 1998). In addition, production of mucilaginous substances and other potentially antiherbivory chemicals may also have decreased the edibility of certain filamentous producers, thereby weakening grazing pressure (Steinman 1996).

Alternatively, lack of statistically significant grazing effects on producer biomass may have been a consequence of low study power. Thus, we performed a post hoc power analysis using a conventional effect size of 0.8 (Quinn and Keough 2002). Based on our level of replication ($n = 3$), power analysis revealed an approximate 55% probability of detecting an 80% difference in autotrophic biomass between our open controls and fine-meshed exclosures. Based on the variance of total biomass responses by producers to the consumer-size treatment, replication would need to be doubled (i.e., $n = 6$ or 12 cages) to detect a significant grazing effect. Morin and Cattaneo (1992) recommended a minimum level of replication of 20 for benthic algal studies using biomass as their response variable, indicating that intense sampling would be required to detect weak grazing effects. In addition, we may have underestimated cyprinid and mollusc biomass because of the D-net sweep method used to sample zoobenthos, which may be reflected in our nonsignificant grazing effects.

Another possibility is that the difference in nutrient concentrations between lake types may have been sufficient to positively affect producer biomass in recovered lakes. Evidence from recovered Killarney lakes has shown that increased producer biomass is positively related to higher TDP (Hogsden and Vinebrooke 2005); however, this is not

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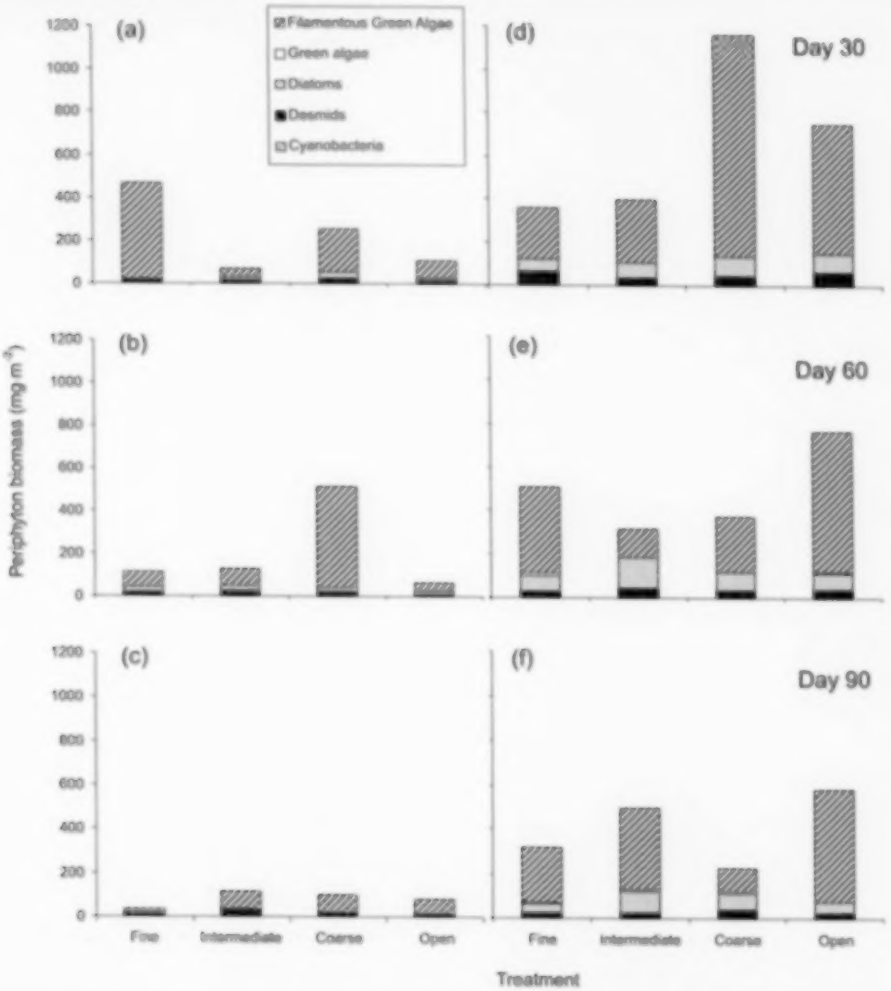
Fig. 5. Benthic producer biomass divided into taxonomic groups found in different exclosure treatments in (*a*–*c*) stressed and (*d*–*f*) recovered lakes over the 90-day experiment.



likely linked to differences in acidification histories, which do not affect TDP levels in boreal lakes (Vinebrooke et al. 2003*a*).

Nevertheless, our ordination analyses suggested that grazers were more abundant in recovered lakes, affecting producers at the genus level. This finding agreed with correlations between body size and feeding selectivity of benthic grazers (France et al. 1991; Vinebrooke 1996; Graham and Vinebrooke 1998). Also, Cattaneo and Kalff (1986) showed that large invertebrate grazers altered benthic producers by selectively feeding on large diatoms and filamentous producers, while not affecting total biomass. In contrast, studies in recovering acidified lakes have shown that high abundance of large benthic grazers can suppress producer biomass (Graham and Vinebrooke 1998). Similarly, Heneberry (1997) reported that the lack of large grazers (e.g., crayfish) resulted in massive accumulation of producer biomass in acid-stressed lakes.

Although planktonic producers exhibit functional compensation in stressed lakes, (Klug et al. 2000; Vinebrooke et al. 2003*b*), acidified lake conditions significantly suppressed benthic producer biomass in our study. The spatial and temporal extent of widespread acidification over several decades may have limited the compensation potential of benthic producers by depleting the regional species pool (Minns et al. 1990; Arnott et al. 2001; Vinebrooke et al. 2003*b*). In contrast, Findlay et al. (1999) and Vinebrooke et al. (2003*b*) have documented recovery by benthic producers from an 8-year experiment in which acid was added to a single lake located among other pristine lakes. Further, small acid-tolerant producers may not fully compensate for larger sensitive taxa, in terms of total biomass (Cattaneo et al. 1998; Klug et al. 2000).

The difference between the impacts of benthic consumers and abiotic stress on producer biomass versus community composition highlight the strong capacity of algae to compensate for weak, natural disturbances (e.g., grazing) but not potentially chronic, novel disturbances (e.g., anthropogenic acidification). Therefore, we must consider interactions among

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Table 5. Summary of repeated measures analysis of variance (RM-ANOVA) and repeated measures multivariate analysis of variance (RM-MANOVA) results including all model effects and interactions.

	Enclosure effectiveness (RM-ANOVA)				Autotrophic biomass (RM-ANOVA)		Chl *a* biomass (RM-ANOVA)				Autotrophic community (RM-MANOVA)			
	Consumer		Grazer		Overall		Overall		Partial cage		Physiognomy		Taxonomic composition	
Effect	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Lake type	0.01	0.94	0.01	0.94	38.36	<0.001	31.40	<0.001	12.35	0.01	44.59	<0.001	26.08	<0.001
Consumer	7.28	0.003	1.08	0.39	0.83	0.51	1.73	0.22	0.01	1.00	0.33	0.81	0.28	0.84
Time	6.21	0.01	5.83	0.01	1.01	0.38	50.37	<0.001	10.56	0.001	22.31	<0.001	49.42	<0.001
Lake type × consumer	0.29	0.84	0.30	0.83	1.01	0.42	1.29	0.33	0.43	0.74	1.40	0.29	0.18	0.91
Lake type × time	0.92	0.41	0.34	0.79	0.27	0.77	5.37	0.01	4.01	0.03	0.78	0.65	7.01	<0.001
Consumer × time	1.34	0.27	1.46	0.23	0.29	0.94	1.50	0.22	0.21	0.97	0.34	1.00	0.39	0.98
Lake type × consumer × time	1.62	0.17	0.70	0.65	0.26	0.95	5.19	0.00	0.48	0.81	0.63	0.94	0.50	0.97

Fig. 6. Association of (*a*) producers and (*b*) lake sampling scores with environmental variables in exclosures in six lakes based on canonical correspondence analysis (CCA) using $\log_{10}$-transformed producer biomass, water chemistry (except pH), and grazer biomass data. For panel *b*, open circles are from recovered lakes, and solid circles are from stressed lakes. DOC, dissolved organic carbon; DIC, dissolved inorganic carbon; TDP, total dissolved phosphorus; and TN, total nitrogen.

abiotic stressors and altered trophic interactions to predict community responses to long-term stress (Frost et al. 1995; Hulot et al. 2000).

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Marine feeding patterns of anadromous brook trout (*Salvelinus fontinalis*) inhabiting an estuarine river fjord[1]

Geneviève R. Morinville and Joseph B. Rasmussen

Abstract: This study describes the ontogenetic and seasonal feeding patterns of anadromous brook trout (*Salvelinus fontinalis*, also known as sea trout) inhabiting the estuarine Saguenay River (Quebec, Canada) using both stomach content and stable isotope analyses. Sea trout of the Ste. Marguerite River (Quebec, Canada) entered the saline waters of the Ste. Marguerite Bay in early May before venturing into the Saguenay River fjord for the remainder of the summer period. Upon their arrival, first-year migrants stayed relatively close to river mouths and initially fed on freshwater aquatic invertebrates. However, they quickly shifted their diet to marine prey items such as amphipods and mysids for the rest of their first summer at sea. These prey items were generally larger than freshwater prey; the prey spectrum at sea was both larger and wider than that found in freshwater and, as such, likely contributed to the trout's rapid growth rates at sea. The diet of migrants in subsequent years at sea (second-year migrants) consisted primarily of marine crustaceans and fish, the latter being most important when feeding in the upper Saguenay River. Trout shifted to piscivory at all marine sites at a size of 25 cm, regardless of time spent at sea, although the importance of piscivory varied with season and site.

Résumé : Notre étude décrit les patrons ontogéniques et saisonniers d'alimentation de l'omble de fontaine anadrome (« truite de mer »; *Salvelinus fontinalis*) qui habite l'estuaire du Saguenay (Québec, Canada) à l'aide à fois de l'étude des contenus stomacaux et de l'analyse des isotopes stables. La truite de mer de la rivière Sainte-Marguerite (Québec, Canada) pénètre dans les eaux salines de la baie de Sainte-Marguerite au début de mai avant de s'aventurer dans le fjord du Saguenay pour y passer le reste de l'été. À leur arrivée, les migrateurs d'un an restent relativement près des embouchures des rivières et se nourrissent au départ d'invertébrés aquatiques d'eau douce. Ils changent cependant rapidement de régime et utilisent des proies marines telles que des amphipodes et des mysidacés pour le reste de leur premier été en mer. Ces proies sont généralement de plus grande taille que les proies d'eau douce; la gamme de proies en mer est aussi plus importante et plus étendue que celle d'eau douce et elle contribue ainsi aux taux élevés de croissance de la truite en mer. Le régime alimentaire des migrateurs des années subséquentes en mer (les migrateurs de seconde année) comprend surtout des crustacés et des poissons marins, ces derniers étant particulièrement importants lorsque la truite de mer se nourrit dans le Saguenay supérieur. Dans tous les sites marins, la truite de mer devient ichtyophage à une taille de 25 cm, quel que soit le temps passé en mer, bien que l'importance de l'ichtyophagie varie en fonction de la saison et du site.

[Traduit par la Rédaction]

Introduction

In many populations of salmonids that access the sea, migrant and resident individuals of the same population coexist as juveniles, with the former spending a portion of their life cycle feeding in saline waters and the latter completing their entire life cycle in freshwater. Those that migrate from freshwater to the sea, returning as large adults to freshwater for spawning, follow an anadromous life cycle. Anadromous brook trout (*Salvelinus fontinalis*, also known as sea trout) naturally occur in northeastern North America in river systems that are open to the sea (Smith and Saunders 1958; Dutil and Power 1980; Castonguay et al. 1982). They generally experience a short but seasonally determined sea residence, returning to freshwater for winter (Dutil and Power 1980; Power 1980), a response most likely necessitated by

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their inability to osmoregulate in cold waters (Saunders et al. 1975). In recent years, angling pressure on sea trout has escalated as returns of Atlantic salmon (*Salmo salar*) to rivers have declined. Unfortunately, few studies involving the anadromous phase of the life cycle have been conducted, limiting our understanding and, thus, ability to implement sustainable management practices.

Juvenile sea trout, before their first seaward migration, exhibit high consumption rates but low growth efficiencies (ratio of growth to consumption) in freshwater compared with residents, the consequence of higher metabolic costs (Morinville and Rasmussen 2003, 2006). The finding that sea trout (migrant) obtain more food in freshwater compared with residents before migration and still migrate suggests that they do not receive enough energy to satisfy their high energetic demands. Juvenile sea trout may thus experience growth bottlenecks sooner than residents, leading them to change habitats in search of an efficient food supply (low cost of capture but high energetic gain). Accordingly, anadromous fish do grow faster in the sea than their resident counterparts of the same age class (Castonguay et al. 1982; Lenormand et al. 2004), suggesting reduced activity costs (swimming, foraging, and other behavioural activities) and (or) better feeding opportunities at sea. Such feeding opportunities may be in the form of differences in overall productivity or differences in the availability of optimally sized prey (Keeley and Grant 2001). Marine systems are generally thought to be more productive for fish than freshwater systems, based on the migratory tendencies of salmonids in north-temperate latitudes (Gross et al. 1988).

In general, fish need to eat large prey to sustain growth and attain large sizes. Foraging costs, both in terms of the time spent actively searching and the number of feeding attempts, increase as prey sizes become small in relation to the size of the predator (Kerr 1971; Kerr and Ryder 1977). Optimal growth returns can thus be easily achieved when the diet is mostly composed of large prey (Kerr 1971; Wańkowski and Thorpe 1979; Sherwood et al. 2002). As such, fish, including salmonids, tend to eat larger prey with increasing size, displaying ontogenetic diet shifts from planktivory to benthivory and ultimately to piscivory (Werner and Gilliam 1984; Mittelbach and Persson 1998; Keeley and Grant 2001). Salmonids tend to eat large prey (fish) sooner in ocean habitats than when inhabiting streams, allowing individuals to attain large sizes more quickly (Keeley and Grant 2001).

Brook trout are commonly considered to be opportunistic feeders, their diet changing with size, season, and habitat (Power 1980). However, few studies report in detail the diet of the anadromous form. Of those that exist, most provide only general descriptions of prey items found in stomachs. These studies commonly report in the diet the presence of amphipods, mysids, and fish, including sand lance (*Ammodytes* sp.), sticklebacks (*Gasterosteus*), smelt (*Osmerus* sp.), and hake (*Urophycis* sp.) (Wilder 1952; Dutil and Power 1980; O'Connell 1982). Only one study has acknowledged the importance of the effect of ontogeny or season on diet of brook trout (Gaudreault et al. 1982), and no study has attempted to directly link diet to the movement patterns at sea. Such information is necessary for implementing sustainable management plans for sea trout, and thus a better understanding of seasonal diet requirements across marine habitats is necessary to, for example, protect the most important feeding grounds.

Lenormand et al. (2004) recently described the ontogenetic and seasonal movement patterns of sea trout migrating to the estuarine Saguenay River (SR) from the Ste. Marguerite River (SMR) in Quebec, Canada. Sea trout of the SMR migrate in May, as early as age 1+, initially entering the saline waters of the SR by passing through the estuarine Ste. Marguerite Bay (SMB), where they remain for a few weeks acclimatizing to the salinities and temperatures of the new habitat, staying relatively close to freshwater inputs (Lenormand et al. 2004). As summer progresses and water temperatures increase, trout gradually migrate out from the bay, with the largest trout leaving sooner to enter the deeper, colder, more saline waters of the Saguenay fjord (SR fjord) (Lenormand et al. 2004). Unlike most anadromous brook trout populations that seem to remain close to the influence of their natal river (White 1940; Dutil and Power 1980), anadromous populations of the SR fjord are unique in that they migrate large distances, upwards of 100 km, experiencing relatively low salinities and high temperatures across the estuary (Chassé and Côté 1991; Lenormand et al. 2004).

The present study investigates the diet of anadromous brook trout immediately upon entering the sea through an estuarine bay. The main objectives of this study were (*i*) to compare the food availability (feeding opportunities) between freshwater and the initial site of sea entry and (*ii*) to describe the feeding patterns of anadromous brook trout during both their first year at sea (hereafter referred as first-year migrants) and their second year or more at sea (hereafter referred as second-year migrants) to better understand the growth patterns experienced at sea. We predicted that the initial site of sea entry, the estuarine SMB, would have more food available than found in freshwater, either in total invertebrate biomass and (or) mean invertebrate size, including a larger range of prey sizes. Seasonal feeding patterns of anadromous brook trout (sea trout) were described across sites in the SR, including the monthly detailed diet of first-year migrants captured in the SMB using stomach content and stable isotope analyses. We expected that sea trout would initially feed on freshwater-derived prey upon sea entry but that they would quickly shift to larger marine-derived prey sources, leading to rapid growth. We also expected that tissues of first-year migrants would become enriched in stable carbon signatures ($\delta^{13}C$) over time because of the enriched $\delta^{13}C$ values of marine prey items compared with those of freshwater (Fry and Sherr 1984). Trout were also predicted to shift to higher trophic levels over time, as indicated by enriched muscle tissue $\delta^{15}N$ signatures.

Materials and methods

Study site

This study was conducted in the SMR system and in the estuarine SR (Quebec, Canada; 48°27′N, 69°95′W; Fig. 1). The SMR flows into the estuarine SR 25 km upstream from the St. Lawrence River maritime estuary (Quebec, Canada). The SMR is home to the largest anadromous brook trout population of the Saguenay River basin (Lesueur 1993). Anadromous brook trout migrate from mid-May to early

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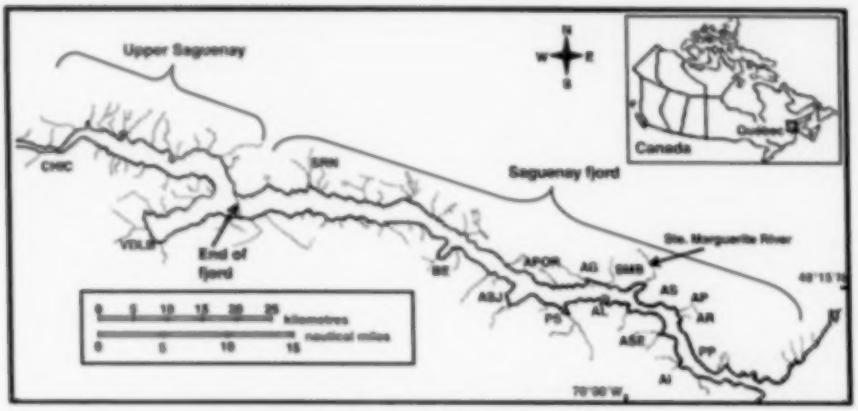
Fig. 1. Map of Saguenay River (Quebec, Canada) sampling sites located in the Saguenay fjord and the upper Saguenay, separated by "End of fjord". Abbreviations: CHIC, Chicoutimi (now City of Saguenay); VDLB, Ville-de-la-Baie (now City of Saguenay); SRN, Ste-Rose-du-Nord; BE, Ste. Eternité Bay; ASJ, Anse St-Jean; APOR, Anse-du-Portage; SMB, Ste. Marguerite Bay; ASE, Anse-St-Étienne; AS, Anse-de-Sable; AP, Anse-à-Pierre; AR, Anse-de-Roche; PP, Pointe à Passe-Pierre; AI, Anse-aux-Petites-Îles; AG, Anse-à-Gagnon; AL, Anse-à-l'Île.



June, as early as age 1+, initially passing through the large, shallow SMB before venturing into the SR (Thériault and Dodson 2003; Lenormand et al. 2004). The SR is divided into two main sections: (*i*) the Saguenay fjord, defined as the last downstream 100 km of the SR under tidal influence and characterized by a mixing zone between freshwater upstream sources and salt-water inputs from the St. Lawrence River maritime estuary, and (*ii*) the upper SR portion. Freshwater inputs into this subarctic fjord are quite variable, with a strong thermohaline stratification occurring between May and October (Chassé and Côté (1991) and references therein; Lenormand et al. 2004). During this period, the water column is divided into a thin, mixed layer (5–10 m) of warm (5–15 °C), brackish (0–18 PSU (practical salinity units)) water and a thick (up to 275 m), underlying layer of cold, saline water (<0.5 °C, >26 PSU at 15 m). The thermohaline stratification regresses in the fall as exchanges between the two layers increase and salinity at the surface rises with decreasing surface temperatures. The ice-cover period occurring between December and April is characterized by surface temperatures around 0 °C and salinity around 7 PSU at the surface. Increasing inputs of freshwater and surface water temperatures during the ice-melt period (mid-March to early May) re-establishes the stratified water column.

Food availability at sea entry

Food availability for both May and August 1998 was compared between freshwater sites located in three tributaries of the SMR (Morin, Allaire, and Épinette; for location of streams, see Lenormand 2003) and 6–7 randomly selected sites located along 30 km of the SMR and multiple randomly selected marine sampling locations (~20 sites) in the SMB, the initial site of sea entry. Sampling was conducted using 1.0 mm mesh kicknets. Sampling in the SMB occurred at tide edges both at high and low tides, depending on location. Some sites were only accessible at low tide. In flowing-water sites (river, stream, and some bay sites), rocks were kicked around in an area corresponding to the width (0.45 m) of the kicknet squared (0.20 m^2) for approximately 30 s, displacing any invertebrates into the kicknet. An average of 10 samples were taken at each site, and all captured invertebrates were pooled for subsequent estimates. In marine sites with no current, the kicker displaced rocks and moved the kicknet in a circle with a radius equivalent to the width of the kicknet (0.64 m^2). This created current and allowed invertebrates to be captured in the kicknet. The same kicker (G.R. Morinville) performed all samplings. All samples were placed in bags and frozen for subsequent taxonomic analyses.

All invertebrates were sorted according to their taxonomic group to the level of order and were counted. Individuals from each taxon were pooled according to their size (size classes in millimetres) when applicable, as determined from the modes of taxon-specific length-frequency distributions. Lengths to the nearest 1 mm were taken using a ruler. This allowed for a more detailed description of the relative contribution of each size class to all classes of a specific taxon for all sites. The relative contribution of each size class was then averaged over all marine and freshwater-specific sites. For the sake of simplicity, only the common taxonomic groups found in the diet of brook trout inhabiting freshwater (mayfly, caddisfly, and stonefly) or the sea (amphipod and mysid) where multiple size classes existed were considered.

Wet weights were measured after the removal of excess moisture with absorptive tissue, and dry weights of samples were taken after samples had dried for 48 h at 60 °C in a drying oven. Food availability was estimated by measuring the mean biomass of pooled invertebrates from each sampling site, expressed as the amount of dry weight of invertebrate prey in grams of dry weight per square metre (g dw·m^{-2}; Boisclair and Leggett 1985) and was compared between freshwater and marine sites using both. Mean invertebrate size (mg dw·individual^{-1}) was also estimated and compared between freshwater and marine sites by pooling the dry weights of all invertebrates within a sample (site) and dividing this value by the total number of individuals in that sample. All comparisons between freshwater and marine sites

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for mean biomass and mean invertebrate size were performed using *t* tests.

Ontogenetic and seasonal feeding patterns

A dual approach using both stomach content analysis (SCA) and stable isotope analysis (SIA) was employed to describe the feeding patterns of anadromous brook trout inhabiting the SR.

Fish collection

Fish stomachs were obtained from trout captured by anglers, from trout obtained for a parallel study (Lenormand 2003; Lenormand et al. 2004), and from additional sampling throughout the SMR, SMB, and multiple SR sites (Fig. 1). At the time of the study, fishing for anadromous brook trout was permitted in the SR, including the SMB, all year round and in the SMR from mid-June to the end of October, allowing for samples from anglers across seasons and sites. Stomachs obtained from anglers throughout the years 1998 to 2001 were available because of a parallel mark–recapture program occurring in the SMR and SMB. Anglers received compensation when they returned tagged fish (Lenormand et al. 2004). Stomachs obtained from trout captured in the SMR during the winter were also made available through this parallel study (Lenormand et al. 2004). Stomachs were either analyzed the day of capture or frozen for future analysis. Both the fork length (to the nearest millimetre) and weight (to the nearest 0.01 g) of sampled trout were measured, although in some cases, this was not possible for trout captured by anglers.

Trout were also sampled in 2000 to 2002 at the onset of sea entry in the SMB in May and every 2–4 weeks thereafter until October using a 40 m beach seine (0.5 cm mesh, 1.5 m deep). In certain years, it was more difficult to catch trout during July and August in the SMB because trout tend to leave the SMB for more saline, colder, deeper areas of the SR with increasing temperatures (Lenormand et al. 2004). Sampling in July and early August was conducted in Anse-de-Sable (AS) and Anse-à-Pierre (AP) (Fig. 1). Approximately 20 trout ranging in size were sacrificed at each sampling interval. Both stomach contents and muscle tissue biopsies (for stable isotopes) were obtained from trout for subsequent diet analyses.

Stomach content analysis (SCA)

The percentage of empty stomachs (%ES) was estimated and used as a measure of feeding activity. Non-empty stomachs consisted of predominantly whole undigested prey items with the exception of freshwater aquatic invertebrate larvae that were sometimes partially digested. Prey items were identified to order or genus for both freshwater aquatic invertebrate and marine prey. Excess moisture was removed from prey items, and like items from each stomach were weighed to the nearest 1 mg (wet weight).

In SR sites (including SMB, upper SR, and Saguenay fjord sites), prey items were subsequently assigned to 10 prey categories: freshwater aquatic larvae, terrestrial insects excluding beetle (coleopteran), beetle, amphipods (mostly *Gammarus* sp.), striped *Gammarus* (*Gammarus tigrinus*), polychaete, mysid, panaeid shrimp, fish, and "other", which includes unidentified prey, plant matter, winged insects including newly emerged flies, and miscellaneous prey items. In the SMR, prey items were assigned to nine prey categories: freshwater aquatic larvae, terrestrial insects excluding beetle, beetle, small mammal, salmonid eggs, winged insects including newly emerged flies, plant matter, fish, and "other", which includes all unidentified prey.

Two stomach content analysis methods were applied to describe the diet composition of anadromous brook trout (Hyslop 1980). The estimates were generated using only the trout having non-empty stomachs. The relative importance of individual prey types was assessed in terms of percentage of occurrence (*%O*) and percentage by weight (*%W*), where the total wet weight of each prey category was expressed as a percentage of the overall weight of stomach contents, termed "prey wet weight contribution" (Clark 1985). *%W* is presented for all prey items in figures, whereas for *%O*, only the two most dominant prey items in fish stomachs are presented in tables.

To describe the ontogenetic and seasonal marine feeding patterns of brook trout, samples were first grouped according to trout type, either first-year migrants (trout in their first year at sea) or second-year migrants (trout in their second or more years at sea). For trout captured in the SMB, stomach contents were pooled by month for years 1998 to 2002, because large samples were available. Monthly means for %ES, *%O*, and *%W* were subsequently generated using pooled years. In contrast, because of limited available captures of brook trout in the SR for any given year and at any given site, monthly %ES, *%O*, and *%W* estimates were obtained by pooling all stomach contents obtained across years at a given site for a given month. These site-specific monthly estimates were further pooled according to their location of capture in the SR, either Saguenay fjord sites or upper SR sites, generating mean monthly estimates. Stomachs obtained from trout captured in the SMR were first pooled by month for years 1998 to 2000, and monthly means for %ES, *%O*, and *%W* were calculated using pooled years.

Stable isotope analysis (SIA)

Carbon and nitrogen stable isotopes ($\delta^{13}C$ and $\delta^{15}N$, respectively) were used to describe the long-term feeding patterns of first-year migrant brook trout from their arrival at sea into the SMB and throughout the summer in relation to the SMB food web. Muscle biopsies were taken from first-year migrant trout captured in the SMB in years 2000 to 2002. Changes in both $\delta^{13}C$ and $\delta^{15}N$ were followed according to the size of migrants and time of capture. The muscle tissue of resident brook trout captured in a tributary of the SMR (Morin Stream) during June and July 1999 were also analysed for stable isotopes, which allowed comparisons between the different regressions with those of sea trout using analyses of covariance (ANCOVA).

To describe the food web leading to sea trout, all potential invertebrate prey items found in the SMB and neighbouring sites downstream to the SMB, including Anse-de-Sable and Anse-à-Pierre, during the years of study were analysed for stable isotopes. Invertebrate prey items, collected using a kicknet, included amphipods (*Gammarus* sp. and *Gammarus tigrinus*), polychaetes, mysids, and freshwater aquatic larvae. Panaeid shrimp were also analysed and captured during seine

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Fig. 2. Mean invertebrate biomass (g dry weight (dw)·m^{-2}) for sites located in the Ste. Marguerite River and Ste. Marguerite Bay in (*a*) May and (*b*) August. No significant differences in invertebrate biomass were found between river and bay sites in either May (t = –0.89, df = 25, p = 0.38) or August (t = –0.22, df = 25, p = 0.83). Broken lines and adjacent numbers indicate mean biomass estimates.

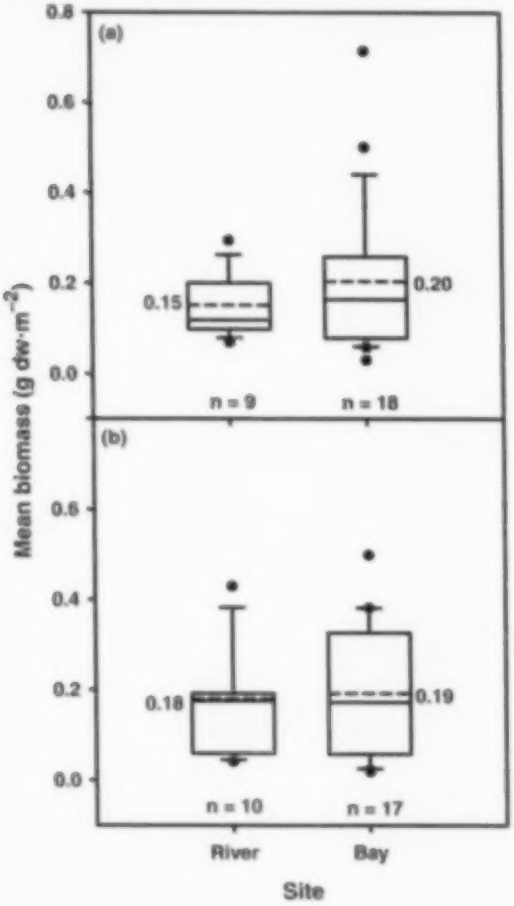
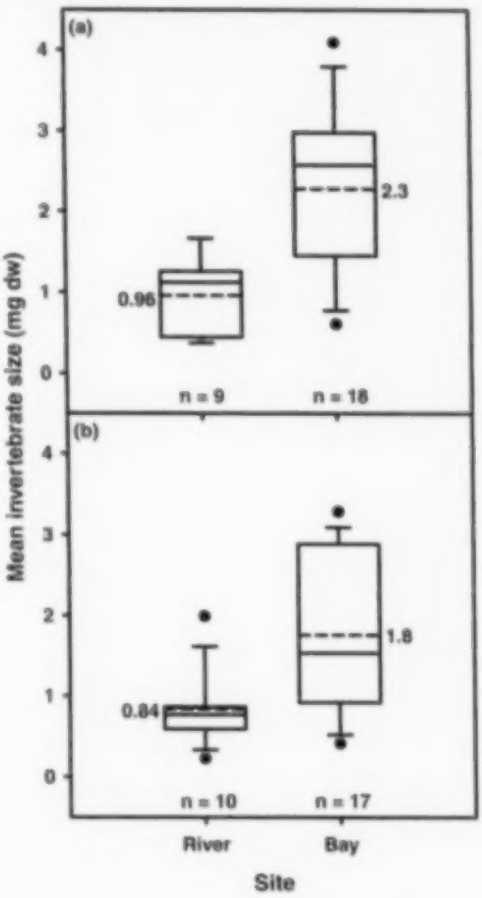


Fig. 3. Mean invertebrate size (mg dry weight (dw)·individual^{-1}) for sites located in the Ste. Marguerite River and Ste. Marguerite Bay in (*a*) May and (*b*) August. River sites had smaller invertebrates than bay sites in both May (t = –3.5, df = 28, p = 0.001; U = 28.0, p = 0.003) and August (t = –2.7, df = 25, p = 0.013; U = 34.0, p = 0.010). Broken lines and adjacent numbers indicate mean sizes.



hauls targeting trout. Fish including threespine sticklebacks (*Gasterosteus aculeatus*) and banded killifish (*Fundulus diaphanus*) were sampled using minnow traps baited with white bread. In addition, muscle biopsies were taken from larger prey fish, including smelt and sand lance, found in the stomachs of certain trout.

Stable carbon and nitrogen isotope analyses were performed using a continuous-flow Finnigan MAT Delta plus mass spectrometer (Finnigan MAT GmbH, ThermoQuest Corp., Barkhausenstr. 2, 28197 Bremen, Germany; G.G. Hatch Isotope Laboratory, University of Ottawa, Ottawa, Ontario, Canada). Stable isotope ratios are normally expressed in delta (δ) notation, defined as the parts per thousand (‰) deviation from a standard material: $\delta^{13}C$ or $\delta^{15}N = [(R_{sample}/R_{standard}) - 1] \times 1000$, where $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$. The standard material is Pee Dee belemnite (PDB) limestone for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$.

Results

Food availability at sea entry

No significant differences were found between river and stream sites for either mean invertebrate biomass estimates or mean invertebrate sizes in either May or August 1998. River and tributaries were thus pooled for all subsequent analyses.

Mean biomass estimates in May 1998 ranged from 0.069 to 0.30 g dw·m^{-2} and from 0.030 to 0.71g dw·m^{-2} for river and bay sites, respectively (Fig. 2). In August, biomass ranged from 0.041 to 0.43 g dw·m^{-2} and from 0.020 to 0.50 g dw·m^{-2} for river and bay sites, respectively. No significant differences in mean biomass were found between river and

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Table 1. Mean contribution (percent of total) of each size class to all size classes of invertebrate taxa obtained during kicknet sampling in freshwater (river) and marine sites (bay) in May and August 1998 for commonly reported prey types found in the diet of brook trout (*Salvelinus fontinalis*).

				May		August	
Site	Invertebrate	Size class	Size-class range (mm)	Numbers[a]	Percent of total[b]	Numbers[a]	Percent of total
River	Mayfly	S	0–4	1106	**56**	734	**70**
		M	5–10	815	42	304	30
		L	>10	18	2	0	<1
	Caddisfly	S	0–8	237	**54**	1215	**56**
		M	9–15	131	44	929	43
		L	>15	12	2	22	1
	Stonefly	S	<10	406	**68**	161	**87**
		M	10–20	174	26	22	6
		L	>20	24	6	7	7
Bay	Amphipod	S	0–5	889	29	1229	27
		M	6–9	1856	**46**	3391	**61**
		L	10–15	921	22	252	11
		XL	16–20	75	2	25	1
		XXL	>20	48	1	3	<1
	Striped Amphipod	S	0–5	92	7	1189	25
		M	6–9	746	37	601	**61**
		L	10–15	1130	**55**	168	13
		XL	16–20	26	1	1	1
		XXL	>20	1	<1	0	<1
	Mysid	S	0–10	895	**83**	49	8
		M	11–20	14	17	3534	**91**
		L	>20	69	0	38	1

Note: S, M, L, XL, and XXL refers to small, medium, large, extra large, and extra extra large, respectively. The size class with the highest overall mean contribution for each invertebrate is bolded.

[a]Numbers shown here do not take into account the density of invertebrates at each site.

[b]Percent of total is the mean contribution of each size class to all classes at each site and averaged over all sites.

bay sites in either May ($t = -0.89$, df = 25, $p = 0.38$) or August ($t = -0.22$, df = 25, $p = 0.83$).

In May 1998, mean invertebrate size ranged from 0.36 to 1.9 mg dw and from 0.60 to 4.2 mg dw for river and bay sites, respectively (Fig. 3). Invertebrate sizes varied in August between 0.23 and 2.0 mg and between 0.40 and 3.4 mg for river and bay sites, respectively. Invertebrates from river sites were smaller on average than those from bay sites for both May ($t = -3.5$, df = 28, $p = 0.001$) and August ($t = -2.7$, df = 25, $p = 0.013$).

Length-frequency distributions (data not shown) of sorted invertebrate taxonomic groups from freshwater sites revealed three size classes (small, S; medium, M; and large, L), ranging in size from 0 to >20 mm in length, for mayfly, caddisfly, and stonefly (Table 1). Across all freshwater taxa, invertebrates comprised mostly the smallest size category (S), ranging from 54% (caddisfly) to 68% (stonefly).

In contrast, five size categories (S; M; L; XL, extra large; and XXL, extra extra large), ranging in size from 0 to > 20 mm, were found for amphipods inhabiting the SMB. A greater number of amphipods were categorized as being medium- or large-sized, evident in both May and August. For mysids, most individuals were small during the month of May; however, by August, medium-sized individuals dominated the size spectrum.

Ontogenetic and seasonal feeding patterns

Stomach content analysis (SCA)

Ste. Marguerite Bay

In the SMB, a total of 972 and 492 stomachs of first- and second-year migrants, respectively, were analysed (Table 2). The mean %ES across months ranged from 8.8% to 20.1% for first-year migrants and from 14.7% to 76.2% for second-year migrants. This amounted to a total of 808 first-year migrants with non-empty stomachs captured between May and October, ranging in size from 114 to 231 mm (Table 3). Second-year migrants with non-empty stomachs (total n = 363) were larger, ranging in size from 220 to 337 mm.

Freshwater aquatic invertebrate larvae and polychaetes contributed the most to the overall wet weight of stomach contents in May, which agrees with the two most common prey items found in first-year migrant stomachs (Fig. 4; Table 4). From June to October, over 50% of the overall wet weight of stomachs consisted of amphipods (*Gammarus* sp.), the most common prey item found in stomachs. In July, terrestrial insects made up an important proportion of the diet, totalling almost 40% of contents. In addition to amphipods, mysids and polychaetes also contributed relatively highly to the diet of first-year migrants from August to October. Prey fish contributed only minimally to the overall weight of the

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Table 2. Number of analysed stomachs (*N*) and percentage of empty stomachs (%ES; ±1 standard error) from first- and second-year migrant brook trout (*Salvelinus fontinalis*) captured in Ste. Marguerite Bay (SMB), the upper Saguenay River (upper) and Saguenay fjord (fjord), and Ste. Marguerite River (SMR) in years 1998 to 2002.

	First-year migrants			Second-year migrants		
Month	Site	*N*	%ES	Site	*N*	%ES
March–May	—	—	—	Upper	17	15.2 (8.8)
April	—	—	—	SMB	5	40
May	SMB	153	13.4 (4.6)	SMB	263	19.1 (2.5)
	—	—	—	Fjord	14	5.0 (5.0)
June	SMB	235	20.1 (6.2)	SMB	106	14.7 (8.0)
	Fjord	14	4.2 (2.9)	Fjord	57	22.9 (15.5)
June–July	Upper	—	—	Upper	5	12.5 (12.5)
July	SMB	72	18.4 (8.5)	SMB	27	15.9 (8.3)
	Fjord	66	9.8 (6.1)	Fjord	32	8.3 (5.3)
August	SMB	60	8.8 (2.5)	SMB	19	39.2 (14.2)
	Fjord	53	22.1 (19.4)	Fjord	13	11.1 (11.1)
September	SMB	283	20.1 (5.8)	SMB	54	38.7 (9.8)
	Fjord	18	6.7 (6.7)	Fjord	6	16.7 (16.7)
	SMR	170	44.7 (10.3)	SMR	204	55.0 (4.2)
October	SMB	169	15.0 (7.8)	SMB	5	20
October–November	Upper	11	46.5 (3.6)	—	—	—
October	SMR	209	56.7 (9.6)	SMR	212	48.6 (2.9)
November	—	—	—	Upper	4	50
December–February	Upper	19	19.4 (10.0)	Upper	18	26.9 (10.9)
January–February	—	—	—	SMB	13	76.2 (9.6)
February	—	—	—	SMR	10	90

contents (less than 5%) and were found in only a fraction of stomachs (5%). Mysids were often found in first-year migrant stomachs, although their contribution to the overall weight of the stomachs was minimal. Most piscivorous individuals exceeded 25 cm in length (Fig. 5). Overall, amphipods consistently contributed the most to the diet of first-year migrants in the SMB across months following the month of May.

As seen for first-year migrants in May, freshwater invertebrate larvae and polychaetes contributed the most to the overall wet weight of stomach contents from second-year migrants, corresponding to the two most dominant prey items occurring in the diet (Fig. 4; Table 4). In June, the diet of second-year migrants was comprised mostly of amphipods (40%) and polychaetes (20%). Contrary to the diet of first-year migrants, fish (all species combined, including sticklebacks, banded killifish, sand lance, and smelt) constituted a large portion of the diet of second-year migrants, but these were mostly found in trout larger than 25 cm (Fig. 5), with %*W* estimates ranging from 17% to 33%. The contribution of amphipods to the diet remained relatively high from July to September, with %*W* estimates ranging from 23% to 59%. Mysids contributed mostly to the diet of second-year migrants in the month of September, with a %*W* of almost 30%. Panaied shrimp occurred frequently in the diet; however, their %*W* was relatively low in comparison with the other prey items.

Saguenay River

Brook trout samples were obtained throughout the Saguenay River, including sites located in the fjord and its upper section. A total of 181 and 166 stomachs of first- and second-year migrants, respectively, were analysed across sites and years (Table 2). Of these, 4.2% to 46.5% of first-year migrants were empty, whereas 5.0% to 50% of second-year migrants were empty. As observed in the SMB, the %ES of both first- and second-year migrants was low during the summer months. Of the trout with non-empty stomachs, 160 were first-year migrants, with mean sizes ranging from 135 to 273 mm (Table 5). Second-year migrants (n = 138) were larger, with mean sizes ranging from 254 to 481 mm.

Amphipods were clearly the most dominant prey item of first-year migrants captured in the Saguenay fjord, in terms of both %*W* (ranging from 50% to 70%) and %*O* (ranging from 54% to 75%) between June and September (Fig. 6; Table 6). Only in June did terrestrial insects contribute strongly to the diet of first-year migrants, comprising 36% of the total wet weight of contents. In contrast, freshwater aquatic invertebrates, terrestrial insects, and fish contributed most to the diet of trout captured in the upper Saguenay sections in November. In winter, prey fish became the dominant item, comprising over 75% of total wet weight. As observed for the SMB, prey fish were mostly found in stomachs of trout larger than 25 cm (Fig. 5).

In the fjord, amphipods and fish contributed the most to the overall wet weight of contents across months, ranging from 26% to 50% and from 16% to 65%, respectively, for second-year migrants (Fig. 6). Terrestrial insects occurred frequently in stomachs, although their overall wet weight contribution was low (Table 6). In August, mysids contributed 34% of the overall wet weight of contents. In contrast, prey fish clearly contributed the most to the overall weight of stomach contents of second-year migrants captured in the

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Table 3. Sample size (*N*) and mean size (±1 standard deviation) of first- and second-year brook trout (*Salvelinus fontinalis*) migrants captured in the Ste. Marguerite Bay (SMB) in May to October in years 1998 to 2002 with non-empty stomachs.

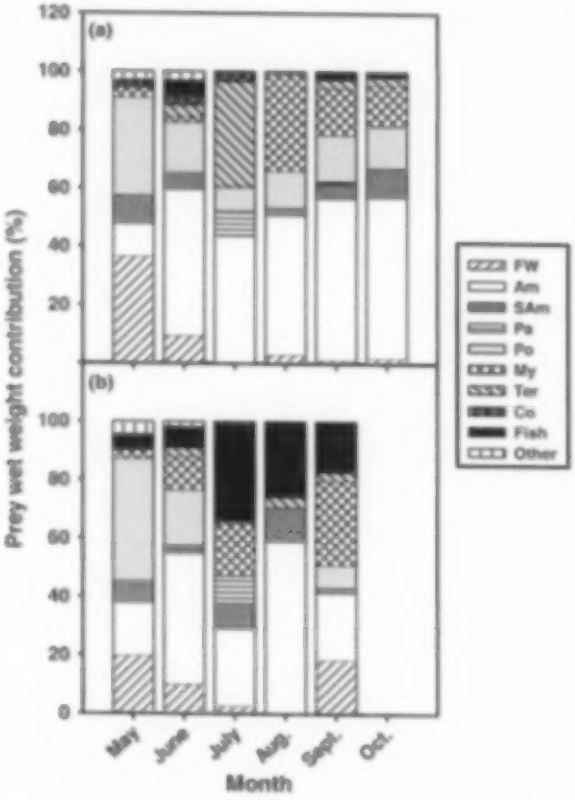
		First-year migrants		Second-year migrants	
Year	Month	*N*	Mean size (mm)	*N*	Mean size (mm)
1998	May	6	148 (14.9)	23	220 (44.5)
1999		12	114 (29.2)	76	240 (44.0)
2000		14	117 (24.2)	31	337 (86.7)
2001		86	118 (25.5)	71	248 (61.9)
2002		6	160 (16.1)	15	229 (32.9)
1998	June	23	177 (25.8)	10	224 (35.7)
1999		33	149 (34.1)	16	231 (40.3)
2000		49	122 (35.5)	47	247 (43.1)
2001		62	118 (28.2)	19	235 (57.6)
2002		19	121 (29.7)	—	—
1998	July	—	—	—	—
1999		9	147 (21.1)	—	234 (18.9)
2000		12	155 (33.0)	15	264 (35.4)
2001		17	122 (20.8)	4	256 (71.9)
2002		22	135 (28.6)	—	—
1998	August	15	212 (13.8)	—	—
1999		25	196 (25.9)	—	266 (24.4)
2000		14	177 (63.6)	4	274 (22.0)
2001		—	—	—	—
2002		—	—	—	—
1998	September	80	231 (32.6)	4	303 (10.6)
1999		78	221 (28.7)	11	275 (30.6)
2000		47	228 (32.1)	18	312 (26.6)
2001		11	201 (21.2)	3	304 (18.0)
2002		22	204 (33.8)	—	—
1998	October	56	228 (22.3)	—	—
1999		39	226 (22.5)	—	—
2000		14	211 (24.2)	—	—
2001		11	191 (25.7)	—	—
2002		26	226 (29.6)	—	—

upper SR, as prey fish %*W* ranged from 60% to 99% throughout the year. Freshwater aquatic invertebrate larvae were important in November, contributing 38% of the overall wet weight of contents. As shown previously, prey fish appeared mostly in trout larger than 25 cm in over 95% of stomachs (Fig. 5); the relative frequency of prey fish found in stomachs of second-year migrants increased significantly with size of second-year migrants ($F_{[1,16]} = 91.2$, $p < 0.001$, $r^2 = 0.85$; Fig. 5).

Ste. Marguerite River

Stomachs of trout from the SMR were obtained mostly in September and October, with the exception of one sampling year when trout were also sampled in February (Table 2). A total of 379 stomachs were obtained from first-year migrants in September and October, whereas 426 stomachs of second-year migrants were analysed, including those in February. For the months of September and October, 44.7% to 56.7% of first-year migrant stomachs were empty. Similarly, the %ES of second-year migrants ranged from 48.6% to 55.0% for September and October, with 90% of stomachs being empty in February. First-year migrants captured in the SMR containing food in their stomachs ($n = 156$) ranged in size from 241 to 262 mm (Table 7). For the second-year migrants captured in the river, 196 had food in their stomachs and ranged in size from 304 to 351 mm.

Fig. 4. Prey wet weight contribution to overall diet of (*a*) first-year and (*b*) second-year migrant brook trout (*Salvelinus fontinalis*) captured in estuarine Ste. Marguerite Bay (SMB) in 1998 to 2002 from May to October. Abbreviations: FW, freshwater aquatic invertebrate larvae; Am, amphipod; SAm, striped amphipod; Pa, panaeid shrimp; Po, polychaete; My, mysid shrimp; Ter, all terrestrial insects excluding coleopteran; Co, coleopteran; "Fish" includes sticklebacks, sand lance, smelt, and killifish; "Other" includes plant matter, winged insects (newly emerged), and all other miscellaneous items.



By wet weight, terrestrial insects made up the largest portion of the diet of first-year migrants captured in September, whereas in October, the diet consisted mostly of freshwater aquatic invertebrate larvae and salmonid eggs (Fig. 7). This is consistent with the two most dominant prey items occurring in the diet during these two months, with the exception of salmonid eggs (Table 8). Similarly, both freshwater aquatic larvae and terrestrial insects were frequently found in stomachs of second-year migrants in September, although the %*W* was highest for terrestrial insects, small mammal, and prey fish remains. In October, both freshwater aquatic invertebrate larvae and salmonid eggs made up a large proportion of the diet, consistent with the diet of first-year migrants.

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Table 4. Number (*N*) of non-empty stomachs and percent occurrence (*%O*) of the two most dominant prey types (prey 1 and prey 2, respectively) found in stomachs (±1 standard error) from May to October (number of pooled years) of first- and second-year brook trout (*Salvelinus fontinalis*) migrants captured in Ste. Marguerite Bay (SMB) in years 1998 to 2002.

	Month	*N*	Prey 1	Prey 1 *%O*	Prey 2	Prey 2 *%O*
First-year	May (5)	124	FW	63.6 (12.7)	Po	31.7 (13.4)
	June (5)	186	Am	44.7 (6.9)	FW	26.4 (8.8)
	July (4)	60	Am	59.8 (15.0)	Ter	49.2 (17.1)
	August (3)	54	Am	66.4 (7.8)	My	48.8 (4.7)
	September (5)	237	Am	71.8 (7.5)	My	28.7 (9.3)
	October (5)	146	Am	68.3 (7.1)	My	28.7 (9.4)
Second-year	May (5)	216	FW	55.7 (14.4)	Po	45.1 (11.3)
	June (4)	91	Am	60.0 (8.6)	FW	24.4 (8.7)
	July (3)	21	Am	56.4 (6.4)	Pa	16.7 (16.7)
	August (2)	11	Am	64.3 (35.7)	SAm	21.4 (21.4)
	September (4)	36	Am	36.7 (15.7)	My	31.5 (5.2)

Note: FW, freshwater aquatic invertebrate larvae; Ter, terrestrial insects excluding coleopteran; Am, amphipod; SAm, striped amphipod; Po, polychaete; My, mysid; Pa, panaeid shrimp.

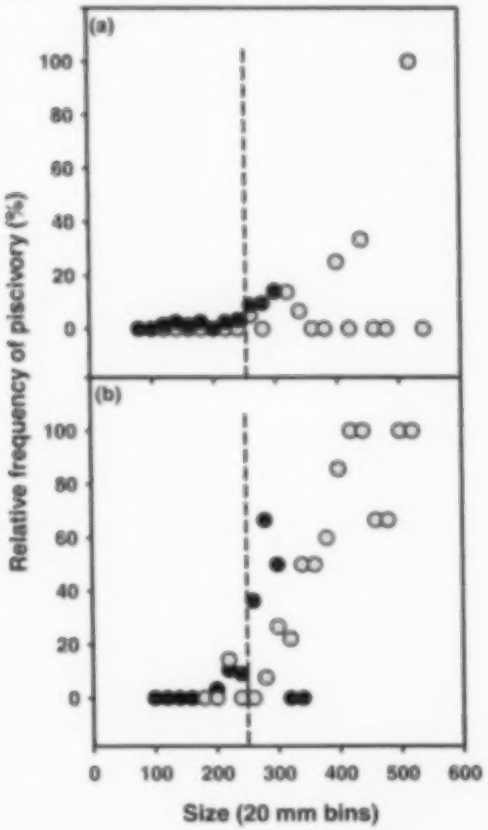

Fig. 5. Relative frequency of piscivory according to size in brook trout (*Salvelinus fontinalis*) migrants captured in (*a*) Ste. Marguerite Bay and (*b*) the Saguenay River. Solid circles and shaded circles refer to first- and second-year migrants, respectively. The broken line separates brook trout above and below the threshold of 25 cm.

Stable isotope analysis (SIA)

A significant positive relationship was found between stable carbon signatures ($\delta^{13}C$) and fork length (FL) of first-year migrants (sea trout) captured in the SMB ($\delta^{13}C = 0.064FL - 29.4$; $F_{[1,74]} = 135.9$, $p < 0.0001$, $r^2 = 0.65$; Fig. 8). Similarly, a relationship was observed between $\delta^{13}C$ and FL for resident brook trout ($\delta^{13}C = 0.0062FL - 25.4$; $F_{[1,38]} = 6.1$, $p = 0.02$, $r^2 = 0.14$). The regressions between $\delta^{13}C$ and FL differed significantly between sea trout and resident brook trout both in slope (ANCOVA: $F_{[1,110]} = 26.5$, $p < 0.005$) and elevation (ANCOVA: $F_{[1,110]} = 6.6$, $p = 0.012$).

Significant relationships were also found between stable nitrogen signatures ($\delta^{15}N$) and FL for sea trout ($\delta^{15}N = 5.2 + 0.036FL - 0.0001FL^2$; $F_{[2,74]} = 18.9$, $p < 0.001$, $r^2 = 0.34$) and for resident brook trout ($\delta^{15}N = 6.0 + 0.0051FL$; $F_{[1,35]} = 4.8$, $p = 0.04$, $r^2 = 0.10$). A significant difference in intercept exists between SMB trout up to 20 cm and resident brook trout (ANCOVA: $F_{[1,108]} = 176.5$, $p < 0.005$).

Marine invertebrate prey items captured in the SMB had signatures ranging from –17.8‰ to –15.6‰ for carbon and from 5.1‰ to 8.5‰ for nitrogen (Fig. 9). Signatures of prey fish, including sticklebacks, banded killifish, and smelt, were enriched in nitrogen compared with the marine invertebrate prey items, ranging from 10.5‰ to 14.0‰. Carbon was slightly depleted in comparison with the invertebrate prey. Freshwater insect larvae had typical signatures observed in freshwater systems. Amphipods obtained from Anse-à-Pierre, a more saline site located downstream of the SMB, had enriched carbon signatures but depleted nitrogen signatures compared with those of the SMB.

Upon sea entry, first-year migrants captured in the SMB in May had mean $\delta^{13}C$ and $\delta^{15}N$ signatures of –25.1‰ and 7.7‰, respectively. By October, trout mean $\delta^{13}C$ and $\delta^{15}N$ signatures rose to –14.5‰ and 9.1‰, respectively, and as expected, nitrogen signatures are 3.3‰ above marine invertebrate prey items, including amphipods and mysids. Sea-age-1 fish had mean $\delta^{13}C$ and $\delta^{15}N$ signatures of –14.7‰ and 9.8‰, respectively, whereas second-year migrants (both adult SMR system spawners and non-spawners) had signa-

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Table 5. Number of non-empty stomachs (*N*) and mean size (±1 standard deviation) of first- and second-year brook trout (*Salvelinus fontinalis*) migrants captured from spring to winter in various sites located throughout the Saguenay Fjord and upper Saguenay River in pooled years 1998 to 2002.

	First-year migrants			Second-year migrants		
Month	Site	*N*	Mean size (mm)	Site	*N*	Mean size (mm)
March	—	—	—	CHIC	2	na
April	—	—	—	CHIC	1	283
May	—	—	—	AR	9	346 (107.8)
	—	—	—	AP	4	274 (93.3)
	—	—	—	VDLB	2	388 (46.7)
	—	—	—	CHIC	8	382 (68.9)
June	AR	11	173 (17.3)	AR	17	303 (98.8)
	AP	2	155 (1.4)	AS	10	254 (43.5)
	—	—	—	AP	7	271 (30.3)
	—	—	—	PS	4	na
	—	—	—	ASE	9	432 (64.9)
	—	—	—	CHIC	3	408 (106.5)
				SRN	2	454 (18.5)
July	ASE	3	180 (16.7)	ASE	8	334 (65.1)
	AI	11	142 (23.9)	AR	7	283 (24.5)
	AR	24	177 (43.9)	AS	8	269 (52.4)
	AS	23	159 (30.1)	AP	6	282 (25.2)
	—	—	—	VDLB	1	481
August	AP	18	164 (29.6)	AS	7	274 (48.7)
	AS	8	198 (29.6)	AP	3	268 (10.0)
	AI	2	214 (20.5)	ASE	2	308 (21.2)
	AL	6	188 (64.9)	—	—	—
	AG	13	135 (9.2)	—	—	—
September	AS	4	201 (29.5)	APOR	2	287 (24.8)
	AI	2	234 (31.8)	AS	2	285 (20.5)
	APOR	11	229 (12.2)	—	—	—
October	CHIC	4	219 (32.1)	—	—	—
November	CHIC	2	191 (27.6)	CHIC	2	356 (5.7)
December	CHIC	3	na	VDLB	1	345
	—	—	—	CHIC	5	365 (27.6)
January	CHIC	9	247 (25.9)	CHIC	2	389 (102.5)
February	CHIC	4	273 (23.2)	CHIC	4	390 (38.4)

Note: na, not applicable. Site abbreviations: AR, Anse-de-Roche; AP, Anse-à-Pierre; ASE, Anse-Ste- Étienne; AI, Anse-aux-Petites-Îles; AS, Anse-de-Sable; AL, Anse-à-l'Île; AG, Anse à Gagnon; APOR, Anse-du-Portage; CHIC, Chicoutimi (now City of Saguenay); VDLB, Ville-de-la-Baie (now City of Saguenay); PS, Petit Saguenay; SRN, Ste-Rose-du-Nord.

tures of about –17.8‰ for carbon and about 12.3‰ for nitrogen.

Discussion

Anadromous brook trout migrating to the SR from the SMR more than double their size over the course of a summer spent at sea (Lenormand et al. 2004). It is expected that for trout to experience such rapid growth rates, the sea must provide better feeding opportunities than freshwater. Such opportunities may come in the form of overall higher productivity and (or) a higher occurrence of large prey (Keeley and Grant 2001), which also includes increased accessibility to such prey. Our results confirmed this prediction, as the SMB, the trout's initial site of sea entry, exhibited better food opportunities (in terms of prey size) than the SMR. Mean invertebrate biomass estimates did not differ between the SR and the SMB in either May or August. However, invertebrates in the SMB were 2.4 times and 2.2 times larger than those found in the SR in both May and August, respectively. Moreover, it was seen that within specific taxonomic groups, a lower proportion of individuals in freshwater sites comprised the larger size classes compared with those occurring in marine sites. Potentially having immediate access to more energetically profitable prey (increasingly larger prey) permits an efficient growth return, because fewer items need to be consumed (and thus captured) to acquire the same amount of energy (Pazzia et al. 2002; Sherwood et al. 2002). Moreover, growth benefits can be materialized more rapidly because the migration, and thus shift to larger prey, follows soon after a period of overwintering and starvation.

Interestingly, a wider range of prey sizes was also observed in the bay versus in the river, indicating that newly arrived fish are presented with a broader range of feeding

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Fig. 6. Prey wet weight contribution to overall diet of (*a*) first- and (*b*) second-year migrant brook trout (*Salvelinus fontinalis*) captured in estuarine Saguenay River (fjord and upper Saguenay) from 1998 to 2002 across months. Abbreviations: FW, freshwater aquatic invertebrate larvae; Am, amphipod; SAm, striped amphipod; Pa, panaeid shrimp; Po, polychaete; My, mysid shrimp; Ter, all terrestrial insects excluding coleopteran; Co, coleopteran; "Fish" includes sand lance and smelt; "Other" includes plant matter, winged insects (newly emerged), and all other miscellaneous items.

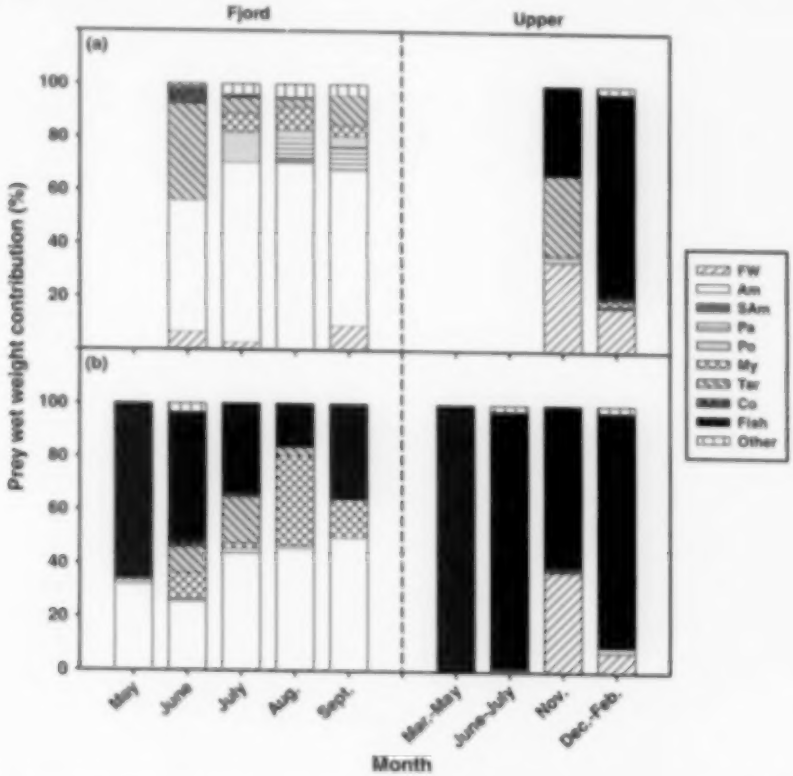

Table 6. Number (*N*) of non-empty stomachs and percent occurrence (*%O*) of the two most dominant prey types (prey 1 and prey 2, respectively) found in stomachs (±1 standard error) from spring to winter (number of pooled sites) of first- and second-year brook trout (*Salvelinus fontinalis*) migrants captured in sites located in the Saguenay Fjord and upper Saguenay River in years 1998 to 2002.

	Site	Month	*N*	Prey 1	Prey 1 *%O*	Prey 2	Prey 2 *%O*
First-year	Saguenay Fjord	June (2)	13	Ter	62.5 (26.5)	Am	54.2 (2.9)
	Saguenay Fjord	July (4)	61	Am	75.0 (6.3)	Ter	16.4 (7.2)
	Saguenay Fjord	August (5)	47	Am	61.9 (12.0)	My	37.8 (8.4)
	Saguenay Fjord	September (3)	17	Am	57.9 (4.1)	Ter	23.3 (14.5)
	Upper Saguenay	October–November (2)*	6	Ter; FW	75.0 (25.0)	—	—
	Upper Saguenay	December–January–February (3)*	16	Fish	65.7 (16.7)	FW	31.5 (11.3)
Second-year	Saguenay Fjord	May (2)	13	Am	52.5 (22.5)	Fish	37.5 (12.5)
	Saguenay Fjord	June (6)	49	Fish	40.1 (18.4)	Ter	30.6 (13.0)
	Saguenay Fjord	July (4)	29	Ter	43.8 (7.7)	Am	41.7 (15.3)
	Saguenay Fjord	August (3)	12	Am	57.1 (29.7)	My	38.1 (31.2)
	Saguenay Fjord	September (2)	4	Am	50 (50)	My; Fish	25 (25)
	Upper Saguenay	March–April– May (4)†	13	Fish	96.9 (3.1)	FW	21.9 (12.9)
	Upper Saguenay	June–July (2)†	4	Fish	100 (0)	FW	33.3 (33.3)
	Upper Saguenay	November (1)†	2	Fish; FW; Co	50	—	—
	Upper Saguenay	December–January–February (4)†	10	Fish	78.8 (14.2)	FW	21.3 (14.2)

Note: FW, freshwater aquatic invertebrate larvae; Co, coleopteran; Ter, terrestrial insects excluding coleopteran; Am, amphipod; SAm, striped amphipod; My, mysid; Fish, includes sand lance and smelt.

*Only includes Chicoutimi (CHIC) site.

†Includes both Chicoutimi (CHIC) and Ville de la Baie (VDLB) sites.

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Table 7. Number of non-empty stomachs (*N*) and mean size (±1 standard deviation) of first- and second-year brook trout (*Salvelinus fontinalis*) migrants captured in Ste. Marguerite River following seaward migration from September to October in years 1998 to 2000.

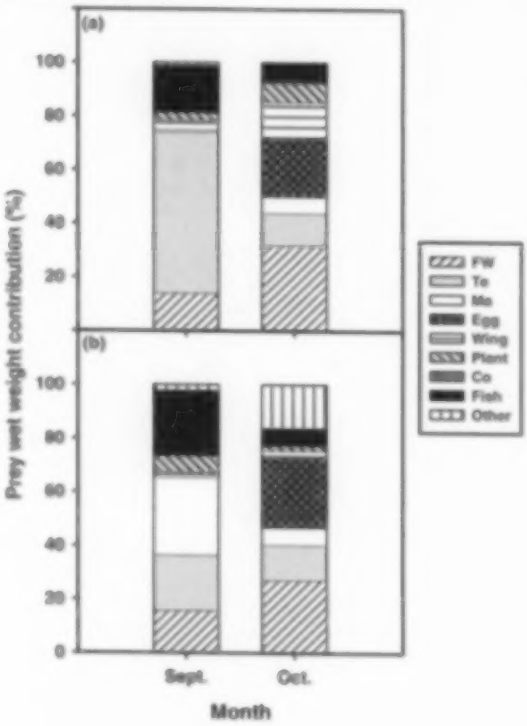
		First-year migrants		Second-year migrants	
Year	Month	*N*	Mean size (mm)	*N*	Mean size (mm)
1998	September	62	256 (26.9)		39
1999		16	249 (27.5)	34	326 (28.6)
2000		6	256 (19.2)	13	304 (37.9)
1998	October	44	256 (19.5)	40	330 (44.0)
1999		23	262 (26.0)	53	341 (44.1)
2000		5	241 (18.8)	17	353 (60.2)

opportunities. The presence of a larger available prey spectrum is also important as it can serve the needs of a wider predator size range and, in turn, limit intraspecific competition for similarly sized prey, because fish generally consume larger prey with increasing size when available (Werner and Gilliam 1984; Keeley and Grant 1997, 2001).

Anadromous brook trout migrating to sea for the first time (first-year migrants) capitalized almost immediately on the better feeding opportunities available in the SMB. Less than 15% of trout sampled in the bay contained an empty stomach, suggesting high feeding activity. Their initial food at sea reflected the transition to a saline habitat from a freshwater habitat. Upon sea entry in May, first-year migrants initially consumed freshwater-derived prey, a conclusion based on the frequency of occurrence and percent wet weight contribution of aquatic insect larvae. This is consistent with earlier reports of high occurrences of aquatic invertebrate larvae also being found in stomachs of brook trout recently entering the sea (Gaudreault et al. 1982; O'Connell 1982). The initial high occurrence of freshwater-derived prey in addition to polychaetes, located mostly in the sand and clay flats surrounding the main river channel outflow (G.R. Morinville, personal observation), agrees with the need of first-time migrants to remain under the influence of the natal river to minimize the costs associated with osmoregulation (McCormick et al. 1985; Lenormand et al. 2004).

By June, first-year migrants inhabiting the SMB rapidly shifted to a diet composed of larger prey, obtaining over 60% of their energy from amphipods and polychaetes within a few weeks of sea entry, consistent with previous reports of feeding behaviours (White 1940, 1942). These amphipods were generally of medium sizes, ranging in length from 5 to 13 mm (G.R. Morinville and J.B. Rasmussen, unpublished data). This feeding pattern was observed in trout exploiting the bay for the remaining summer months and early fall, where at least 50% of the energy was derived from amphipods in addition to prey items such as mysids and polychaetes. The frequent ingestion of mysids later in the summer suggests a surface-oriented feeding position close to shore (Montgomery et al. 1990). Amphipods in addition to sand lance were also the dominant prey items in trout sampled between late August and early October in the Matamek River estuary (Whoriskey et al. 1981). These observations were also consistent with earlier studies involving the anadromous

Fig. 7. Prey wet weight contribution to overall diet of (*a*) first- and (*b*) second-year migrant brook trout (*Salvelinus fontinalis*) captured in Ste. Marguerite River from 1998 to 2000 in September and October. Abbreviations: FW, freshwater aquatic invertebrate larvae; Ter, all terrestrial insects excluding coleopteran; Ma, small mammals; "Egg" includes eggs of either brook trout or Atlantic salmon (*Salmo salar*); "Wing" includes newly emerged flies and adult flies; "Plant" includes all plant matter; Co, coleopteran; "Fish" includes sand lance and smelt; "Other" includes plant matter, winged insects (newly emerged), and all other miscellaneous items.



form of brown trout (*Salmo trutta*) and Arctic char (*Salvelinus alpinus*) (Moore and Moore 1974; Pemberton 1976; Knutsen et al. 2001). Other than a few occurrences of sticklebacks and sand lance in the spring and fall, fish were infrequently encountered in stomachs of first-year migrants captured in the SMB. Similarly, second-year migrants, having spent at least one previous summer at sea, returned to the deeper waters of the Saguenay fjord, passing through the SMB during the month of May. As with first-year migrants, they initially fed on polychaetes and aquatic invertebrate larvae during their descent into the bay, although by June, their diet was composed mostly of marine crustaceans (amphipods).

First-year migrants that ventured into the Saguenay fjord also had a diet composed mainly of amphipods, contributing over 50% to their diet, in addition to terrestrial insects and mysids. These trout acquired an orange–pink flesh over the course of the summer, confirming a diet composed of carotenoid-rich marine crustaceans (Peterson et al. 1966). No piscivorous first-year migrants feeding in the fjord (not

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Table 8. Total number (*N*) and percent occurrence (*%O*) of the two most dominant prey types (prey 1 and prey 2, respectively) found in stomachs (±1 standard error) from September to October (number of pooled years) of first- and second-year brook trout (*Salvelinus fontinalis*) migrants captured in the Ste. Marguerite River (SMR) in years 1998 to 2002.

	Month	*N*	Prey 1	Prey 1 *%O*	Prey 2	Prey 2 *%O*
First-year	September (3)	84	Ter	60.3 (24.0)	FW	42.0 (13.4)
	October (3)	72	FW	68.7 (8.4)	Plant	16.9 (10.1)
Second-year	September (3)	86	FW	59.9 (6.7)	Ter	23.1 (8.3)
	October (3)	110	FW	52.4 (11.7)	Ter	23.6 (8.9)

Note: FW, freshwater aquatic invertebrate larvae; Ter, all terrestrial insects excluding coleopteran; Plant, includes all plant matter.

Fig. 8. Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) signatures as a function of fish length (FL) for sea trout (anadromous brook trout (*Salvelinus fontinalis*); solid circles) captured in the Ste. Marguerite Bay ($\delta^{13}C = 0.064FL - 29.4$, $p < 0.0001$, $r^2 = 0.65$; $\delta^{15}N = 5.2 + 0.036FL - 0.0001FL^2$, $p < 0.001$, $r^2 = 0.34$) and for resident brook trout (open circles) captured in Morin Stream, a tributary of the Ste. Marguerite River ($\delta^{13}C = 0.0062FL - 25.4$, $p = 0.02$, $r^2 = 0.14$; $\delta^{15}N = 6.0 + 0.0051FL$, $p = 0.04$, $r^2 = 0.10$). Sea trout were captured from May to September. Resident trout were captured in June and July.

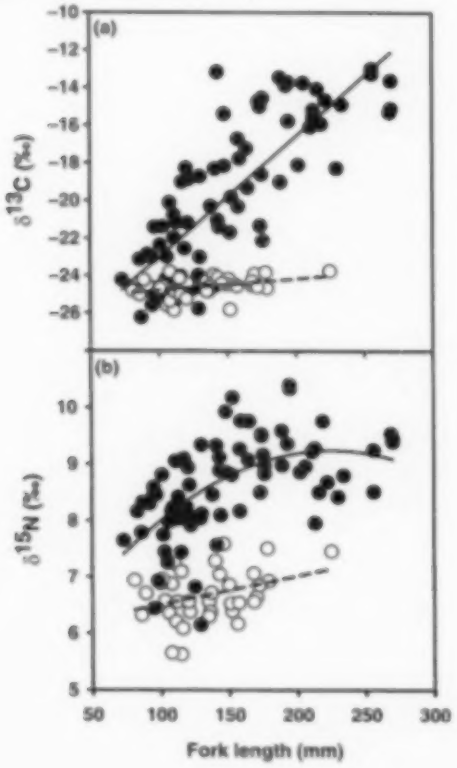
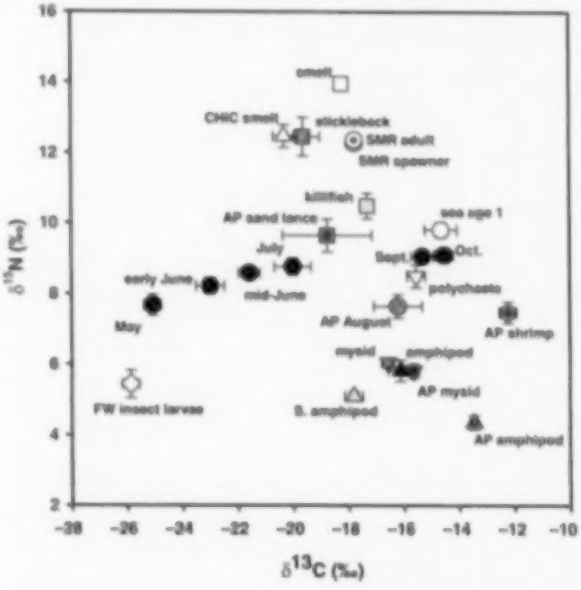


Fig. 9. $\delta^{15}N$ and $\delta^{13}C$ of anadromous brook trout (sea trout, *Salvelinus fontinalis*) from the Ste. Marguerite River system, Quebec. May, early and late June, July, September (Sept.), and October (Oct.) indicate the mean signature of first-year sea trout captured in the Ste. Marguerite Bay (SMB) during those months. "AP" August and "AP" prey items refer to isotope signatures of first-year migrants and prey items captured in Anse-à-Pierre (Saguenay River), respectively, which is located downstream of the SMB. Sea-age-1 refers to sea trout captured in the SMB in early May that are beginning their second summer at sea; FW, freshwater; S., striped; SMR spawner and adult refers to an anadromous brook trout spawner and nonspawner captured in the Ste. Marguerite River; CHIC smelt, smelt found in the stomach of a trout captured in CHIC (upper Saguenay River site).



including the SMB or upper SR) were ever encountered in the 5 years of study. This contrasts with the diet of second-year migrants inhabiting the fjord and upstream regions of the SR. Similarly, fish made up only a minor component of the diet of first-year Arctic char, brown trout, and St. John River brook trout migrants, whereas second-year migrants fed more heavily on fish (Gaudreault et al. 1982; Rikardsen and Elliott 2000; Knutsen et al. 2001).

The rapid shift to marine prey items in first-year migrants was clearly observed with stable isotope signatures of trout muscle tissues. Between May and October, the $\delta^{13}C$ signature of first-year migrants increased by more than 10‰ from a freshwater signature of –25‰ to a more marine signature of –14.5‰. This change was detected with increasing trout size. As expected, such abrupt changes in $\delta^{13}C$ were not ob-

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served in resident brook trout with increasing size. The diet of trout remaining in freshwater streams, as revealed by stomach content analyses, indicated no important change of diet with ontogeny because trout continued to feed mostly on freshwater aquatic invertebrates and some terrestrial insects (G.R. Morinville, unpublished data).

δ^{13}N signatures of first-year migrants also changed with size, increasing from 7.7‰ to 9.1‰ by October. Slight increases were also observed in resident brook trout, possibly reflecting a shift to larger and slightly higher level prey items, such as predatory caddisfly. In the SMB, amphipods had δ^{13}C and δ^{15}N signatures averaging –16‰ and 5.8‰, respectively, whereas mysids had signatures averaging –16.6‰ and 6.1‰, respectively. Given the expected fractionation between prey and predator of about 0‰–1‰ for carbon and about 3‰–4‰ for nitrogen (DeNiro and Epstein 1978; Minagawa and Wada 1984; Peterson and Fry 1987) and the information obtained from stomach content analyses, amphipods and mysids appeared to be the main prey items from which first-year migrants obtained their energy.

Trout (first-year migrants) that migrated to the upstream sections of the SR to overwinter in freshwater had a diet consisting primarily of fish and freshwater aquatic invertebrate larvae in late autumn, followed by a diet made up almost entirely of fish (mostly smelt) in the winter months. Similarly, Knutsen et al. (2001) also reported higher occurrences of fish in the diet of anadromous brown trout during autumn. In this study, the shift to piscivory occurred when sea trout reached sizes of at least 20 cm, with a relative frequency of piscivory of 50% occurring at 25 cm. Prey fish also predominated in stomachs of sea trout larger than 30 cm in length captured in coastal waters of Newfoundland, Richmond Gulf (Quebec), and the St. John River (Quebec) (Dutil and Power 1980; O'Connell 1982). Pemberton (1976) also found that brown trout larger than 21 cm had higher occurrences of fish in their diet than those smaller than 21 cm, which consumed primarily crustaceans and insects.

Trout migrating to the upper Saguenay remained actively feeding (less than 20% empty stomachs), instead of returning to their natal river, obtaining high-energy returns throughout the winter months and allowing them to maintain their condition. In contrast, those returning to their natal river during fall decreased their feeding activity (50% empty stomachs), feeding mainly on small, low-energy prey, including terrestrial insects and aquatic invertebrate larvae, consistent with the diet of anadromous brown trout returning to freshwater (Harris 1971). Traces of fish (either trout or salmon) and small mammals, in addition to salmonid eggs, were noted in a fraction of stomachs, supporting the observation of their opportunistic feeding behaviour (Power 1980), although sea trout still tended to be of poor condition upon their return to sea following winter (Lenormand et al. 2004).

Interestingly, trout having spent the previous summer at sea and captured the following spring had a similar δ^{13}C signature as those captured at the end of October, indicating no change in feeding habits or habitats over winter. The slightly heavier δ^{15}N signature in comparison with the October trout may reflect a starvation effect leading to enrichment (Vander Zanden and Rasmussen 2001), inferred from the observation that trout descending the river in spring are of lower condition than those that overwintered in and continued to feed in the upper SR (Lenormand et al. 2004). The effect of starvation may also explain the 1‰ difference in δ^{15}N observed between first-year migrants and residents at small sizes because differences in diet during their coexistence in streams are unlikely.

Although δ^{15}N of first-year migrants increased with size during the course of the summer, the change was not linear. Instead, it followed a curvilinear pattern with increasing size, reaching a high of around 20 cm in length and decreasing slightly thereafter. This is expected to occur following a rapid shift in diet because tissue signatures approach new prey signatures asymptotically over time, because of the time lag resulting from the residual pool of tissues with the old signature. Moreover, the non-linear pattern may also be reflecting changes in feeding location. As mentioned earlier, trout during the summer exited the SMB and migrated to neighbouring sites across the Saguenay, including the downstream Anse-à-Pierre and Anse-de-Roche sites. First-year migrants initially captured in the SMR system have been frequently recaptured in these locations (Lenormand et al. 2004). As indicated by SIA, migrants continued to feed heavily on marine amphipods at these sites and throughout the Saguenay fjord. Marine amphipods collected in AP were found to be more enriched in δ^{13}C but lighter in δ^{15}N compared with those in the SMB. It is thus quite likely that trout, when feeding outside of the SMB and in the fjord, acquired the signature of amphipods found in sites such as Anse-à-Pierre before their return to the SMB in the fall, leading to the observed curvilinear pattern with increasing size. This is likely as trout (not necessarily originating from the SMR system) captured in August in Anse-à-Pierre had lower δ^{15}N signatures than those captured in the SMB in July.

It is important to mention that second-year migrants captured in the SMB from July to September were likely returning from earlier migrations to the greater SR. These trout were possibly future spawners (Lenormand et al. 2004) and may have started reducing their energy intake upon return to the bay, although some may have continued eating amphipods and fish. This has been previously reported in sea trout populations of the Moser River (Nova Scotia), in which over 50% had empty stomachs, and of those with contents, 27% were almost empty (Wilder 1952). Indeed, the percentage of empty stomachs of second-year migrants was highest in the bay during the months of August and September, reaching levels of almost 40%. Those that continued upstream into the SMR presumably further reduced their feeding activity, because over 50% of captured trout had empty stomachs in the fall. Over 80% of sea trout likely to spawn and captured on the spawning grounds also had empty stomachs (G.R. Morinville and J.B. Rasmussen, unpublished data). Similarly, White (1940) also reported no food in the stomachs of ascending trout. In winter, over 75% of second-year migrants captured in the SMB or SMR had empty stomachs, suggesting older migrants in winter virtually ceased feeding until the following spring. This reduced feeding behaviour may explain the low condition factor of trout descending the river in early spring (Lenormand et al. 2004).

Anadromous brook trout have previously been considered largely piscivorous (Power 1980), although these conclusions

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were based on earlier reports of feeding that failed to consider the size at which piscivory begins or whether fish eating occurred on a seasonal basis. For all sampled habitats, piscivorous trout generally exceeded 25 cm in length, consistent with other salmonid populations (Keeley and Grant 2001). This threshold of piscivory was consistent regardless of whether trout were in their first or more years at sea. Moreover, high occurrences of frequent piscivory generally occurred in specific locations of the Saguenay River, notably the upper SR (CHIC and VDLB sites), and during specific seasons, winter and spring, with smelt being the dominant fish species in trout stomachs (G.R. Morinville, personal observation). In winter, smelt may be more susceptible to predation by trout as the activity of smelt is lowered under ice cover (Vinni et al. 2005), whereas in the spring, smelt migrate upstream for spawning (Pigeon et al. 1998). Seasonal dependence on fish in the diet has also been reported in anadromous brown trout, where fish were by mass the dominating prey during winter feeding, likely reflecting prey availability during those months (Knutsen et al. 2004).

Stable isotope signatures of SMR adult migrant spawners and non-spawners indicated that the sea trout never fully became piscivorous on smelt. This was inferred from the dual isotope approach in which migrants had lighter $\delta^{13}C$ (difference of 2.5‰) but similar $\delta^{15}N$ signatures to those of the upper SR smelt. If smelt had contributed fully to the diet of sea trout, we would have expected trout to reach a $\delta^{15}N$ signature of about 3.4‰ above the smelt signature, given the fractionation between predator and prey (Minagawa and Wada 1984; Vander Zanden and Rasmussen 2001); however, this was not observed. Muscle tissue $\delta^{15}N$ signatures of sea trout from the SMR system seldom go beyond 14‰ (G.R. Morinville and J.B. Rasmussen, unpublished data). Such signatures were largely supported by SCA as second-year migrants continued to feed on amphipods and mysids during the summer across the SMB and fjord. Sea trout of the St. John River (Quebec) were also reported to feed heavily on amphipods throughout the year, even at large sizes, although fish contributed highly to the summer diet (Gaudreault et al. 1982). Alternatively, migrants could also be feeding heavily on sand lance, as $\delta^{15}N$ signatures of sand lance were approximately 2.8‰ lower than those of adult migrants.

Few competitors are present where brook trout enter the sea. Both sticklebacks and banded killifish can be found in the SMB, with sticklebacks being the most abundant permanent resident species of the bay (Mousseau and Dodson 1996). However, threespine sticklebacks in the bay are relatively small (5–8 cm FL; M. Bélanger and J.B. Rasmussen, unpublished data). Both SCA and SIA indicated that sticklebacks captured in the SMB between May and July consumed mostly marine amphipods and freshwater aquatic invertebrate larvae, depending on their proximity to freshwater (M. Bélanger and J.B. Rasmussen, unpublished data). First-year migrants were also found to eat such prey items, although migrants entering the SMB were, on average, larger than sticklebacks, ranging in size from 7 to17 cm (Lenormand et al. 2004). Given their larger size and the wider prey spectrum available in the bay, it is unlikely that trout strongly competed with sticklebacks for the same prey size categories. However, stronger competitive interactions could develop over time if trout were to migrate at increasingly smaller sizes, a situation that could arise following increasingly poor growth conditions in freshwater before migration.

In conclusion, marine crustaceans, mostly amphipods, were the most important prey item in the diet of first-year migrants, allowing them to experience rapid growth rates during their first summer spent at sea. During their ontogeny, migrants, whether they were in their first year at sea or greater, shifted their dependence to prey fish at the threshold size of 25 cm. First-year migrants generally reached these sizes after their first summer of growth, allowing them to benefit from energy-rich fish (mostly smelt) in the first winter, provided they migrated to the upper SR for winter. The highest levels of piscivory occurred in the upper SR sites, signifying this region as an essential feeding ground for sea trout, allowing for continual growth. Of concern, the agglomeration of sea trout in these feeding grounds during winter and early spring makes sea trout especially vulnerable to overfishing and habitat destruction. As a consequence, strict regulations need to be implemented, including both the protection of feeding grounds and limits to fishing during these months. Additional regulations protecting the prey base of sea trout also need to be implemented to preserve the anadromous form.

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Baseline expression of heat-shock proteins (HSPs) of a "thermotolerant" Mediterranean marine species largely influenced by natural temperature fluctuations

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Abstract: Northwestern Mediterranean warming-related disease outbreaks and species shifts have recently been documented. Biomarkers of short-term effects on the health or resistance of organisms are necessary to assess and understand mechanisms affecting marine biodiversity. Heat-shock protein (HSP) expression was here believed to present finer variations than previously reported in experimental studies, which we tested to assess HSP sensitivity and relevance in the global warming context. One population of the "thermophilic" marine cave mysid *Heteromysis margalefi* was monitored over 4 years. Three HSPs presented recurrent expression. Among them, HSP50 and HSP60 expressions varied seasonally with environmental temperature. HSP60 expression displayed the greatest variability and was most closely related to temperature fluctuations. Multiple regression showed that while the main stressor was temperature, demographic variables (proportion of immatures, proportion of gravid females) also influenced expression variability. Such a monitoring of HSP baseline expression in a natural population had only rarely been conducted. HSPs are, however, good biomarkers to evaluate the effects of warming episodes or heat stress in the wild. This study provides essential spatial and temporal reference for further experimental and in situ analyses and is a prerequisite to diagnostic and predictive approaches.

Résumé : Avec le réchauffement de la Méditerranée nord-occidentale, des apparitions de maladies et des remplacements d'espèces ont récemment été décrits. Des biomarqueurs d'effets à court terme sur la santé ou la résistance des organismes sont nécessaires pour évaluer et comprendre les mécanismes affectant la biodiversité marine. Les protéines de choc thermique (HSP) pourraient présenter, comme on le suppose dans ce travail, des variations plus fines que précédemment rapportées dans des travaux expérimentaux. Leur sensibilité et leur utilité dans le contexte du réchauffement global sont évaluées sur une population naturelle du mysidacé « thermophile » de grottes sous-marines *Heteromysis margalefi* suivie pendant 4 ans. Trois HSP montrent une expression récurrente. Parmi elles, les expressions des HSP50 et HSP60 varient saisonnièrement avec la température ambiante. L'expression de la HSP60 montre la plus grande variabilité et est plus étroitement liée aux fluctuations de température. Une régression multiple montre que bien que la température soit le principal facteur de stress, certaines variables démographiques (la proportion d'immatures, la proportion de femelles gravides) influencent aussi la variabilité de l'expression. Un tel suivi de l'expression de base d'HSP dans une population naturelle a rarement été entrepris. Les HSP sont cependant de bons biomarqueurs pour évaluer les effets des épisodes de réchauffement ou du stress thermique dans la nature. Cette étude fournit un point de référence spatio-temporel pour de futures analyses expérimentales et in situ, et constitue un pré-requis à des approches diagnostiques et prédictives.

Introduction

Organisms are subject to multiple stress factors with antagonistic or combined effects (Sørensen et al. 2003) occurring at different levels of biological organisation. Global warming is one of the major threats on biodiversity, with demonstrated effects on growth and production, phenology, species distribution, and species interactions (Hughes 2000; Harvell et al. 2002). Much less is known about short-term effects occurring at lower biological levels.

Despite their ecological relevance, indices of affection at the highest levels of biological organisation only give a

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delayed assessment of the effects of a global perturbation and thus cannot detect early signs of stress. For ecological risk assessment, predicting the effects of long-term perturbations (e.g., global warming) requires biomarkers of short-term effects reflecting the mechanisms by which the health of organisms is altered (Lagadic 2002). Long-term and large-scale monitoring of resistance biomarkers, rather than their punctual experimental use, can also provide more accurate information on the intrinsic resistance capabilities of organisms and (or) populations and on how they counterbalance environmental stresses in the wild.

Heat-shock proteins (HSPs) are involved in maintaining homeostasis, with constitutive expression in normal conditions and over-expression during or after environmental stress. They respond to a wide range of stress factors (temperature, pollutants, pathogens), making them biomarkers of environmental stress (Sørensen et al. 2003). However, there are very few in situ applications, particularly for monitoring the effects of heat stress in natural environments or as a correlate of a warming trend (some examples given by Hofmann and Somero 1995; Minier et al. 2000). Most published works study the influence of an acute or repetitive stress on the physiology of organisms raised or stressed in the laboratory. Although such approaches are helpful in providing specific biological responses to a given (controlled) stress, they are less representative and less sensitive than fieldworks. Therefore, they are not sufficient to adequately assess the combination of biotic and abiotic factors found in natural conditions. To account for stressors at play in normal conditions, the natural baseline expression of the biomarker must be determined, at least over one generation or over one year for long-lived species. In the present context of global warming, this is of fundamental importance to estimate and quantify its ecological and evolutionary consequences on organisms and populations (Somero 1995; Sørensen et al. 2003).

In the Mediterranean, a warming trend with a higher frequency of exceptional meteorological–hydrological events have been documented (Béthoux and Gentili 1999; Salat and Pascual 2002). Biological effects such as northward migrations of thermophilic species, disease outbreaks, and mass mortalities inducing local extinctions or species shifts have been reported (e.g., Pérez et al. 2000; Chevaldonné and Lejeusne 2003). A species shift between two cave-dwelling mysids (Crustacea, Mysidacea) could be related to the more thermophilic characteristics of *Heteromysis margalefi*, which could successfully outcompete its congener *Hemimysis speluncola* (Chevaldonné and Lejeusne 2003; Lejeusne and Chevaldonné 2005). Since the severe 1999 thermal anomaly, *H. margalefi* has been dominating most dark marine caves of the northwestern Mediterranean and was chosen as the biological model of the present work.

Until now, climate warming has been studied at population or community level. Here we offer a better understanding of such phenomena at the organism level, using HSPs (integrated over a long time) as biomarkers of the resistance ability of organisms. We hypothesized HSP expression presents finer seasonal variations than previously believed, allowing us to (*i*) apply HSPs for the first time as biomarkers in a long-term monitoring of natural populations, (*ii*) test their expression sensitivity to natural fluctuations of temperature, and (*iii*) test their relevance as thermal stress markers in the context of global warming.

Material and methods

Biological model

Hemimysis margalefi is a cave-dwelling crustacean (Crustacea: Mysidacea) that forms swarms of hundreds to thousands of individuals in the darkest parts of caves of the northwestern Mediterranean. With the warming of the Mediterranean and the related increase in thermal anomaly frequencies, it has recently become the dominant cave-dwelling mysid of the northwestern Mediterranean, replacing its congener *H. speluncola* (Chevaldonné and Lejeusne 2003). As any mysid species, it is characterized by the incubation of larvae into a marsupial pouch, and reproduction occurs year round with one more intensive recruitment period at each season (Lejeusne and Chevaldonné 2005). Its demographic characteristics and thermal stress tolerance experiments highlighted the thermotolerant affinities of this species, making *H. margalefi* a particularly interesting biological model for the present work.

Sampling

Mysids were sampled between May 2000 and July 2004 by SCUBA using diver-operated, specially designed suction bottles (squirt bottles fitted with a nozzle, two valves, and a 400 µm mesh filter) to avoid damage to samples. The large cave of Jarre Island (43.2°N, 5.4°E, 15–17 m depth) was chosen as the collection site because of the year-round presence of an abundant swarm.

Collected mysids were identified and examined (sex, size, demographic category, marsupial content) in vivo under a dissection microscope as soon as their arrival in the laboratory (see Lejeusne and Chevaldonné 2005 for details). To minimize transportation- and examination-induced stress, individuals were transported in a large volume of cave water in an insulated box to the lab, then kept in a temperature-controlled room at 15 °C in the dark always in the original seawater from the cave until their individual examination. After examination, they were individually frozen at –80 °C for protein preservation.

Western blot analysis

Protein extraction was performed using 80 mg of each replicate (see below) suspended in 800 µL TTBS (Tris–HCl 100 mmol·L^{-1}, NaCl 0.9%, Tween 20 0.1%, phenyl-methyl-sulfonyl fluoride 1 mmol·L^{-1}, pH 9.5) and centrifuged 90 min (19 000*g*; 4 °C). Supernatant was then collected and stored at –20 °C. A part of this supernatant was used to determine the total protein concentration according to the Lowry method (Lowry et al. 1951).

The remaining supernatant was diluted five times in a treatment buffer (0.06 mol·L^{-1} Tris–Cl, 2% sodium dodecyl sulfate (SDS), 10% *v/v* glycerol, 0.1 mol·L^{-1} dithiothreitol, 0.01% bromophenol blue, pH 6.8) and boiled 5 min. Proteins were separated in 8% polyacrylamide minigels during 2 h (35 mA) using a Mini-Protean® 3 Electrophoresis Cell (Bio-Rad Laboratories, Hercules, California). Proteins were then electrotransferred to nitrocellulose membranes (Bio-Rad

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Laboratories) at 0.8 mA·cm^{-2} for 1 h in blotting buffer (25 mmol·L^{-1} Tris, 192 mmol·L^{-1} glycine, 20% methanol, 0.1% SDS, pH 8.3) using a semidry blotting apparatus (Mini Trans-Blot Cell®, Bio-Rad Laboratories). Membranes were blocked overnight in TBS with 3% bovine serum albumin at room temperature.

The monoclonal antibody (McAb), anti-bovine brain HSP70 (anti-HSP70 Ab, raised in mice; Sigma–Aldrich Corp., St. Louis, Missouri) (H5147) and secondary antibody (anti-mouse Ig-G) alkaline phosphatase conjugate (Promega Corporation, Madison, Wisconsin) were used for the immunoblotting. Membranes were incubated with McAb (1/1000 dilution, 2.4 μg·mL^{-1}) in Tris-buffered saline with 0.15% Tween 20 for 90 min at room temperature, followed by incubation with the secondary antibody (1/1000 dilution, 1 μg·mL^{-1}) for 90 min at room temperature. The immunoblot was stained using Western Blue™ stabilized substrate for alkaline phosphatase (Promega Corporation).

A high molecular weight standard mixture for SDS gel electrophoresis (molecular weight range 30 000 – 200 000; Sigma–Aldrich Corp.) was used to determine the weight of the proteins revealed by immunoblotting, and a purified HSP70 from bovine brain was used to quantify them.

Blots were digitized and band analysis was performed with the QuantityOne® software (Bio-Rad Laboratories). The intensity of each band was quantified and divided by the intensity of the purified HSP70 deposited on each blot, thus allowing comparison among different blots.

For each sampling date, three replicates of seven pooled individuals were used. Preliminary tests showed that this replicate size was the best to obtain the proper protein concentration efficient for immunoblotting.

Temperature records

To study the relation between HSP expression and seasonal variations of temperature, we obtained temperature time series from the study area. We did not have a complete time series from the Jarre station over the 4 years studied, but a temperature-recording station existed on a nearby island of the Riou archipelago, located only a couple of kilometres from Jarre cave (depth: 11 m; station setup by J.G. Harmelin, Station Marine d'Endoume, UMR 6540 DIMAR, Rue de la Batterie des Lions, F-13007 Marseilles, France), which we used from 11 January 2000 to 31 October 2002. From 1 November 2002 to 31 August 2004, the Jarre cave was equipped with its own temperature recorder at 15 m. Seamon® mini temperature loggers (Hugrún, Iceland) were used with recording intervals of 3 h. To avoid daily variability in temperature recording that could induce bias in the biological interpretation of the relationship, values used in the present work correspond to the mean of temperatures recorded during the 7 days preceding the sampling date (sampling day measures included).

Statistical analysis

The relationship between variations of temperature and expression of each of the identified HSPs was assessed using Spearman's rank correlation (R_S) and Kruskal–Wallis nonparametric rank-sum tests (H). However, to also study the potential influence of others factors on HSP expression and the magnitude of their respective contribution to protein expression, a multiple regression was conducted. HSP expression was used as the dependent variable (after log-transformation to meet assumptions of normality; Shapiro–Wilk test, $P > 0.05$) and abiotic (temperature) and biotic factors obtained from demographic data collected for each replicate as independent variables (Table 1). However, multicolinearity between independent variables must be avoided in multiple regression. This was addressed by examining correlation coefficients (r) derived from a Pearson's correlation matrix. When $|r| \geq 0.5$, variables were considered as correlated (Table 2). This procedure left five among the nine initial independent variables: temperature, mean size of *H. margalefi* individuals, sex ratio (defined as the ratio of mature males vs. mature and gravid females), proportion of immature (including juveniles and immature individuals), and proportion of gravid females among mature females (see Lejeusne and Chevaldonné 2005 for details on the demographic categories). To eliminate the effects of colinearity, Mallows' C_p was used for each HSP as a criterion to select the best model (with the lowest C_p) from a set of models containing uncorrelated combinations of those five latter variables. Consequently, sets of resulting variables were included in the multiple regression using backward stepwise selection.

Results

HSP expression

The McAb raised against bovine homologous HSP70 immunoreacted in *H. margalefi* revealing three recurrent bands with molecular masses of ~45, ~60, and ~85 kDa (Fig. 1). They will subsequently be referred according to the HSP families to which they belong: the widely known HSP50, HSP60, and HSP90. This confirms the high degree of conservation of the HSP multigenic family.

Each HSP presents variation in its expression over the sampling period. HSP50 presents the lowest levels of expression: from nondetectable (20 March 2001 and 21 March 2003) to 0.041 ± 0.021 μg·μg total protein^{-1} (mean ± standard error (SE); 23 August 2000). HSP60 presents the highest levels of expression (0.011 ± 0.001 μg·μg total protein^{-1}, 3 May 2004 to 0.079 ± 0.029 μg·μg total protein^{-1}, 23 August 2000), whereas HSP90 presents intermediate levels (0.005 ± 0.005 μg·μg total protein^{-1}, 4 December 2000 to 0.054 ± 0.006 μg·μg total protein^{-1}, 1 August 2000). However, ranges only represent the extreme variations of the different HSPs and do not allow a direct comparison. This can be done using coefficients of variation. They show that HSP60 present extremely variable expression levels (72.19%) over the whole sampling period, whereas HSP50 and HSP90 vary somewhat less (62.42% and 58.04%, respectively).

Temperature records

The time series obtained give an overview of the seasonal variations of temperature in the 10–15 m depth range in the Marseille area. Data from the Riou time series present the same overall pattern as data from Jarre cave and are thus suitable for our subsequent analyses. The four seasons are easily identified on the underwater records. The period from

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Table 1. Demographic parameters of the *Hemimysis margalefi* samples.

Sampling date	Mean size (mm)	SR	Imm./Mat.	F_{whole}	M_{whole}	F_g/F_{whole}	M	F_g
2000								
24 May	4.85	1.1	0.24	0.48	0.52	0.86	0.52	0.29
01 August	4.6	1.1	0.05	0.48	0.52	0.67	0.52	0.29
23 August	4.32	1.25	0	0.42	0.56	0.25	0.56	0.11
08 September	4.39	0.62	0	0.62	0.38	0.92	0.38	0.57
16 October	4.41	0.82	0.17	0.52	0.43	0.56	0.43	0.24
09 November	4.74	0.75	0.05	0.57	0.43	0.91	0.43	0.48
04 December	4.52	1.25	0.4	0.38	0.48	0.5	0.48	0.19
2001								
12 February	4.87	0.75	0.31	0.57	0.43	0.5	0.43	0.24
20 March	4.37	0.67	0.75	0.57	0.38	0.25	0.38	0.10
06 April	5.26	0.5	0.05	0.67	0.33	0.79	0.33	0.52
03 May	5.79	0.75	0	0.57	0.43	0.17	0.43	0.10
2002								
21 May	5.33	0.33	0.05	0.71	0.24	0.2	0.24	0.14
25 October	4.76	0.5	0	0.67	0.33	0.79	0.33	0.52
26 November	4.26	0.64	0.62	0.52	0.33	0.22	0.33	0.10
2003								
20 January	4.85	1.25	0.11	0.38	0.50	0.63	0.5	0.25
21 February	5.44	5.67	0.05	0.14	0.85	0.5	0.85	0.05
21 March	3.00	—	19.00	0.00	0.35	—	0.35	0
22 April	4.57	3.5	0.75	0.19	0.67	0.75	0.67	0.14
27 May	3.91	3.5	0.62	0.19	0.67	0.25	0.67	0.05
23 June	3.93	1.25	0.62	0.38	0.48	0.71	0.48	0.24
24 July	4.55	0.62	0.11	0.62	0.38	0.5	0.38	0.29
21 August	4.45	0.24	0	0.81	0.19	0.65	0.19	0.52
29 September	4.68	0.27	0.17	0.71	0.19	0.64	0.19	0.43
05 November	4.6	1.86	0.17	0.33	0.62	0.43	0.62	0.14
26 November	4.7	1.22	0.17	0.43	0.52	0	0.52	0
2004								
07 January	4.95	1.5	0.11	0.38	0.57	0.38	0.57	0.14
10 March	4.78	3	0.31	0.24	0.71	0.2	0.71	0.05
04 May	5.21	4.25	0	0.19	0.81	0	0.81	0
15 July	4.73	1.63	0	0.38	0.62	0.38	0.62	0.14
Total	4.65	2.37	0.2	0.46	0.48	0.53	0.48	0.23

Note: SR, sex ratio; Imm./Mat., ratio immature/mature; F_{whole}, proportion of females (including mature and immature); M_{whole}, proportion of males (including mature and immature); F_g/F_{women}, proportion of gravid females among mature females; M, proportion of mature males; F_g, proportion of gravid females.

December to February displays the coldest records with extremely low variations (range: 12.30–16.07 °C; mean ± standard deviation (SD): 14.08 ± 1.15 °C); March to May corresponds to a slight warming (range: 12.13–19.40 °C; mean ± SD: 14.02 ± 1.25 °C), whereas June to August presents the warmest records (up to 26 °C) and typically the highest variability with occasional sudden decreases (down to 13.48 °C) of temperature over periods of a few hours or days because of the northwestern "Mistral" wind regime (Pérez et al. 2000; Romano et al. 2000). This implies a relatively low mean temperature with an important variation (mean ± SD: 17.76 ± 3.02 °C). Finally, the period September to November also records elevated temperature, but the duration of stable periods (usually in October) seems to be longer than in summer (range: 13.55–23.13 °C; mean ± SD: 18.34 ± 2.37 °C).

No major differences among recorded years or thermal anomalies at considered depths have been noted in these records. However, we noted that year 2000 was the warmest on average (16.37 ± 3.20 °C), whereas 2001 was the coldest year (15.33 ± 2.85 °C). The mean value over the whole recorded period was 16.10 °C ± 2.92 °C.

Relationship between HSP expression and recorded temperatures

A linear regression of each HSP expression data set with in situ recorded temperature shows that a significant, positive relationship exists for HSP50 and HSP60 (Spearman's R_S rank correlation coefficient: $R_S = 0.241$, $P < 0.05$ and $R_S = 0.369$, $P < 0.05$, respectively; Fig. 2). As a consequence, the warmest year of the sampling period (2000) presents the highest expression levels of HSP60 and HSP50. HSP90 does not present a significant relationship between its expression and temperature records ($R_S = 0.128$, $P > 0.05$). As shown with HSP60 (Fig. 3), HSP50 and HSP60 also present maximal expression during the warmest (but also the most variable)

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Table 2. (*i*) Matrix of pairwise correlations among independent variables used for the multiple regression and (*ii*) β coefficients of the influencing variables in the backward procedure.

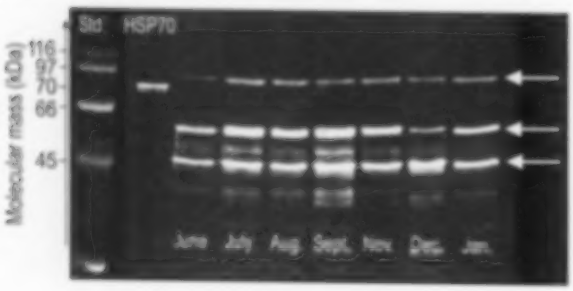
Variable	Temperature	Size	SR	Imm./Mat.	F_{whole}	M_{whole}	F_g/F_{mature}	M
Correlation among independent variables of the multiple regression								
Size	–0.35							
SR	**–0.32**	0.03						
Imm./Mat.	**–0.21**	**–0.44**	–0.003					
F_{whole}	0.33	0.10	**–0.79**	–0.09				
M_{whole}	**–0.31**	0.03	**0.81**	–0.09	**–0.94**			
F_g/F_{mature}	**–0.10**	0.18	–0.06	0.11	–0.004	0.03		
M	–0.20	0.19	**0.77**	**–0.32**	**–0.80**	**0.90**	0.01	
F_g	0.09	**0.24**	**–0.40**	–0.10	**0.39**	**–0.35**	**0.81**	**–0.32**
β coefficients								
HSP50[a]	**0.3742**		**0.3556**					
HSP60[b]	**0.3979**						**–0.3225**	
HSP90								

Note: Bold numbers are significant values at the level $\alpha \leq 0.05$. SR, sex ratio; Imm./Mat., ratio immature/mature; F_{whole}, proportion of females (including mature and immature); M_{whole}, proportion of males (including mature and immature); F_g/F_{mature}, proportion of gravid females among mature females; M, proportion of mature males; F_g, proportion of gravid females. Note that none of the studied variables could explain variations in the expression of the HSP90 using a backward stepwise procedure in the multiple regression analysis.

[a] $r^2_{adj} = 0.2087$, $P = 3 \times 10^{-3}$.

[b] $r^2_{adj} = 0.2699$, $P = 2 \times 10^{-4}$.

Fig. 1. Western blot (inverted picture) of heat-shock proteins (HSP) in *Heteromysis margalefi*, samples from June 2003 to January 2004. From top down, HSP90, HSP60, and HSP50 are marked by arrows on the right. Std, standard molecular mass; HSP70, pure HSP70.



period of each year (summer and early autumn) and minimal expression during the coldest and most stable period (January to May). Expression of these proteins is therefore closely related to the seasonal variations of temperature (Kruskal–Wallis test, $H_{(3,90)} = 11.45$, $P = 0.009$ for HSP50 and $H_{(3,90)} = 13.06$, $P = 0.004$ for HSP60). However, the simple linear regression analysis showed that only 5% ($R^2 = 0.049$, $P = 0.004$) and 8% ($R^2 = 0.083$, $P = 0.007$) of the variation in the expression of HSP50 and HSP60, respectively, could be explained by the temperature fluctuations (Fig. 2).

A multiple regression analysis was conducted to investigate whether addition of demographic variables could improve the understanding of HSP expression variations. Temperature and the proportion of immature individuals were the two variables selected for HSP90 and HSP50 (Mallow's $C_P = 2.36$ and 1.56, respectively), whereas temperature, sex ratio, and proportion of gravid females among mature females were selected for HSP60 ($C_P = 3.18$). Using a backward procedure, this analysis (Table 2) allowed us to greatly improve the proportion of variance explained for HSP50 (21%) and HSP60 (27%). However, in both cases, temperature remained the main variable influencing the expression of both proteins. HSP50 expression was also positively influenced by the proportion of immature individuals, whereas a greater proportion of gravid females among mature females tended to decrease HSP60 expression (β coefficients in Table 2). On the contrary, none of the studied variables could explain variations in the expression of the HSP90 using a backward stepwise procedure in the multiple regression analysis.

Discussion

The heat-shock response is one of the major responses to protect cells from protein injuries caused by various stress factors. An overview of the studies conducted on HSP induction in some metazoans is given, and it shows the lack of in situ applied studies of this biomarker (Table 3). Moreover, very few studies have already reported seasonal changes in stress proteins expression, and they were only focusing on a comparison of two dates or periods of the year, or they only monitored changes over a short period of a few months during only 1 year (e.g., Anneli Korhonen and Lagerspetz 1996; Choresh et al. 2001; Robbart et al. 2004). These works did not take the biological characteristics of studied organisms into account, such as generation time and related life history, either because they were unknown or because the analysis was conducted on reared individuals, thus representing natural, biological reactions in a simple or biased perspective. Bias due to artificial life conditions (e.g., acclimation) may result in disturbances at the cellular level and may affect many life history and fitness traits, such as fecundity, longevity, and stress resistance (Sørensen et al. 2003). Laboratory experiments cannot reflect the true base-

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Fig. 2. Correlation between heat-shock protein (HSP) concentration and temperature. Significant values ($\alpha = 0.05$) are indicated with asterisks. Note the vertical scale change for the different panels. (*a*) HSP50; (*b*) HSP60; (*c*) HSP90.

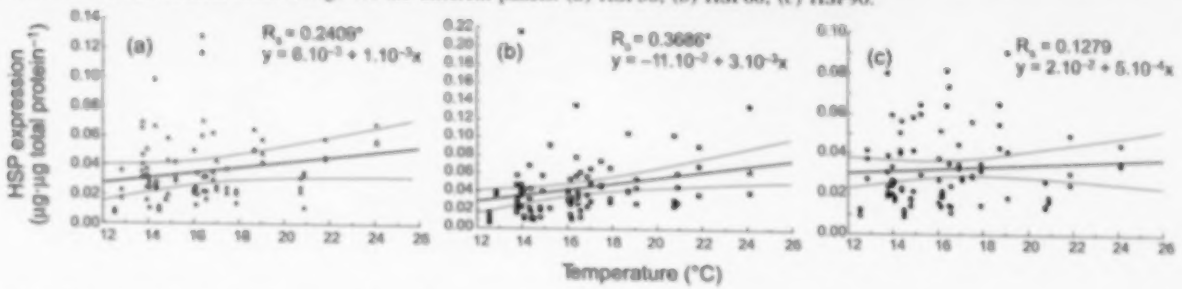
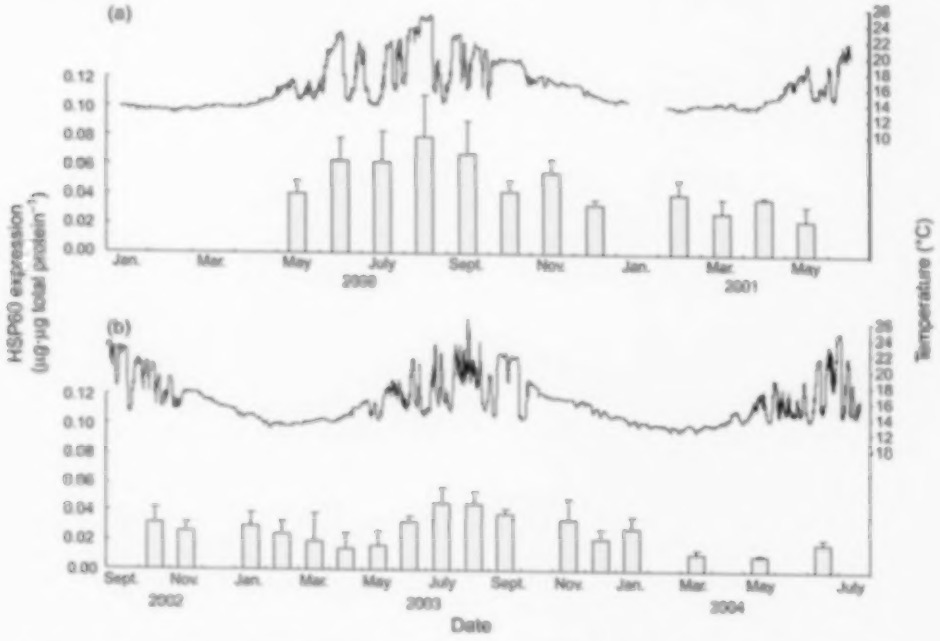


Fig. 3. Seasonal variations of the HSP60 expression in *Hemimysis margalefi* and of temperature during the periods (*a*) May 2000 – May 2001 and (*b*) October 2002 – July 2004. Histogram bars correspond to the mean of three replicates at a given date with the corresponding standard error.



line expression patterns of wild populations, which actually require spatial–temporal monitoring studies of stress markers in the wild. Our sampling strategy, based on a natural population that has generated 16 cohorts over the 4 sampled years (Lejeusne and Chevaldonné 2005), provides for the first time in the marine environment a real opportunity to assess the combined effects of a given environmental stress and some biotic factors. Indeed, working on a single population located in a chemically homogeneous environment allowed us to focus on the effects of natural temperature fluctuations.

Temperature as a main stress factor

Three HSPs have shown recurrent expression in all replicates and samples over the whole sampling period. Among them, HSP60 and HSP90 belong to the best-studied families. Both are constitutively expressed as chaperon proteins but are also involved, among other things, in steroid receptor complexes (Lindquist and Craig 1988; Parsell and Lindquist 1993; Sørensen et al. 2003). The HSP50 family is comparatively less known (Table 3). However, this family, as well as the HSP60 family, has been previously reported as constitutive and thermo-inducible proteins (e.g., Parsell and Lindquist 1993; Feder and Hofmann 1999; Choresh et al. 2001).

In *H. margalefi*, HSP50 and HSP60 expressions are positively correlated with seasonal changes in environmental temperatures no matter what year was considered. HSP60 presents the greatest seasonal changes in expression, and its variations are also the most closely related to seasonal changes in temperature. In summer, HSP60 expression can be up to three times higher than in winter (see 2003 in Fig. 3). HSP50 shows the same trend, but differences between seasons appear less pronounced. Although we cannot directly compare protein quantities, variations across seasons are of the same order than

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Table 3. Stressors inducing a heat-shock protein (HSP) expression in some invertebrate phyla with corresponding family involved in the response.

Phylum	Stressor	HSP family (kDa)	No. of in situ studies	Reference
Porifera (sponges)	Heat shock, PCB, TBT, heavy metals, combined stressors	50, 70	3	Batel et al. 1993; Müller et al. 1995; Koziol et al. 1996, 1997; Müller and Müller 1998; Schröder et al. 1999; Agell et al. 2001; Pérez 2001
Cnidaria	Heat shock, seasonal temperature, competitor, PCB, combined stressors	20–30, 60, 70, 90	1	Sharp et al. 1994; Black et al. 1995; Downs et al. 2000; Wiens et al. 2003; Rossi and Snyder 2001; Robbart et al. 2004; Rossi et al. 2006
Mollusca	Heat shock, seasonal temperature, PCB, TBT, Cd	70	3	Sanders 1988; Ellis and Pattisina 1990; Steinert and Pickwell 1993; Brown et al. 1995; Smerdon et al. 1995; Lundebeye et al. 1997; Bradley et al. 1998; Chapple et al. 1998; Minier et al. 2000; Hofmann and Somero 2005; Sorte and Hofmann 2005
Urochordata (tunicates)	Heavy metals, combined stressors	70	1	Agell et al. 2004
Arthropoda	Temperature, Cd, Pb, desiccation, molt cycles	50, 70, 90	1	Bradley et al. 1992; Powell et al. 1992; Bond et al. 1993; Gehring and Wehner 1995; Asnelli Korhonen and Lagerspetz 1996; Werner and Nagel 1997; Feder et al. 1999; Knigge and Kobler 2000; Cimino et al. 2002; Ravaux et al. 2003; Schill et al. 2003; Hayward et al. 2004; Voznesensky et al. 2004; Chang 2005; McMillan et al. 2005

Note: The number of in situ applied studies corresponds to a large search in the literature, whereas given references are not exhaustive but are representative of current HSP research with an ecological perspective. TBT, tributylin.

in the few marine metazoans whose HSP's seasonal expressions have been reported: the mussels *Mytilus edulis*, *Mytilus galloprovincialis*, and *Mytilus trossulus* (HSP70) (Hofmann and Somero 1995; Chapple et al. 1998; Minier et al. 2000) and the anemone *Anemonia viridis* (HSP60) (Choresh et al. 2001). In all cases, HSP expression variations over time have been strongly correlated with temperature variations.

Within our sampling period, the highest maxima of temperature were recorded in summer 2003 (up to 26 °C), which was also a period of very high air temperatures and a year with a sea-surface positive thermal anomaly in the northwestern Mediterranean area (Harmelin 2004). However, summer 2000 was the warmest summer of our sampling period, and year 2000 as a whole was the warmest on average (16.37 ± 3.20 °C). This discrepancy reflects in part the fact that the cave was located below the maximum depth (15 m) impacted by the 2003 thermal anomaly. *Hemimysis margalefi* in fact exhibited the highest expression levels of HSP50 and HSP60 during summer 2000 (significantly higher than in summer 2003), which shows how sensitive and integrative such biomarkers can be. The response is extremely fine, since any minor temperature change between seasons or between years can be detected. The variations observed in HSP expression between seasons and between years also demonstrate that they are expressed during periods of reduced thermal stress, such as winter. The higher expressions during summer suggest that more protein damage occurs with higher temperatures than during cooler seasons.

The potential relationship between HSP90 expression and thermal stress is not clearly established. This HSP family is referred to as a stress-inducible protein in a number of organisms, including crustaceans (Black et al. 1995; Chang 2005), but we failed to confirm these results. With an experimental study on HSP90 and HSP70 mRNA synthesis, Chang (2005) showed a lower expression for HSP90 than for HSP70 after heat stress. According to this author, HSP90 expression is tissue-specific and depends on various factors such as osmotic stress. On the other hand, this protein family does not seem to be required for tolerance to extreme temperatures in yeast (Parsell and Lindquist 1993). All this could explain why we could not find a correlation between this HSP and seasonal variations of temperature. A hypothesis to be tested would be the possible involvement of HSP90 in the response of crustaceans to acute heat-shocks rather than chronic exposures.

Influence of some secondary factors

We demonstrated that two secondary factors of biological origin influence the HSP expression levels of *H. margalefi*. Associated with temperature, the percentage of immature individuals and the percentage of gravid females among females better explained the expression variability of HSP50 and HSP60, respectively. The expression of HSP50 is higher in *H. margalefi* samples with the highest percentages of immatures. Many species have distinctive patterns of HSP expression across their development stages (gametogenesis, embryogenesis, metamorphosis), depending on their sex and age class (e.g., Feder and Hofmann 1999; Sørensen et al. 2003; Chang 2005). Therefore, HSPs are ecologically relevant for all life stages (Feder and Hofmann 1999; Sørensen et al. 2003). For example, non-adult *Drosophila melanogaster*

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produce a massive heat-shock response when infesting necrotic fruits (extremely warm if sunlit), or encysted *Artemia* embryos, undergoing a development arrest, accumulate enormous concentrations of a small HSP (26 kDa) (Feder et al. 1997; Liang et al. 1997). In the present work, besides the major influence of temperature on HSP50 expression, it may preferentially be expressed in immature stages of *H. margalefi*. A shift might occur later with the preferential expression of another HSP (possibly HSP60) in mature stages. However, a common pattern in HSP expression during developmental stages is that no expression can occur during the initial phases of embryogenesis or during gametogenesis, possibly because of some deleterious aspects of HSP that are harmful to developing cells (see Feder and Hofmann 1999). Therefore, we hypothesize that the observed negative effect of the percentage of gravid females on the expression of HSP60 in *H. margalefi* may be due to the lack of expression of this protein in the embryos and larvae carried by females in their marsupium. This would apparently decrease the concentration of HSP detected compared with the total protein concentration. This idea is reinforced by the high values of the ratio of gravid females in most of the replicates.

Other potential biotic or abiotic factors could also greatly influence the expression levels of the three HSPs considered. Pollution may influence stress protein levels, but the good and stable water quality of our study site has previously been demonstrated (Pérez et al. 2003). Among natural abiotic factors, pH, salinity, dissolved O_2, and light intensity variations may also induce HSP. Such environmental factors were not considered in the present study, but their potential influence on HSP expression could explain part of the unresolved variance observed. However, these parameters are usually reported to be rather stable in marine caves (Fichez 1990). The stress proteins expressed in *H. margalefi* coincide in time with the summer–autumn trophic crisis that could also constitute a stress source, as suggested for another Mediterranean marine invertebrate (Rossi et al. 2006). Other factors such as sex and age of collected individuals have previously been reported to influence HSP expression (e.g., Solé et al. 2004; Chang 2005). In the case of *H. margalefi*, sex does not significantly influence HSP expression. However, despite all such possible factors, the main stressor at play in HSP expression has always been demonstrated to be temperature (Sørensen et al. 2003).

Assessing the effects of warming

In the present context of global warming of the Mediterranean Sea and of the increasing frequency of extreme climatic events, temperature is a key stress factor in an ecological and biological conservation perspective. Owing to anthropogenic activities including and resulting in, among others, global warming, pollution, and habitat fragmentation, environmental changes could become more drastic and their biological effects much more difficult to predict in the future. This is why the development of an early warning system for benthic communities is of particular interest, whatever the ecosystem considered, at tropical, temperate, or boreal latitudes (Smith and Buddemeier 1992; Somero 1995). Biomarkers at the individual and population levels appear particularly useful as both diagnostic and predictive tools (Lagadic 2002). Such biomarkers must be applicable to any biological model whatever the phylum and the maturity stage considered, and a strong background must exist on their response type to natural stress (severe or not). Monitoring their baseline is the first and fundamental step for diagnostic and predictive perspectives, since it establishes a spatial and temporal reference for further experimental and in situ analyses. Providing the baseline expression of the biomarker on model and non-model organisms then shows how representative of a considered community it may be.

The originality of the present work is to have studied the stress induced by natural, seasonal variations of temperature rather than a permanent or acute stress. We define natural stress as any stress due to biotic or abiotic factors whose frequency, magnitude, and duration can be encountered in the natural environment. Natural stress can thus be chronic, such as seasonal variations of temperature or a regular input of pollutants, or rare and acute, such as thermal anomalies or oil spills. On the contrary, experimental stress refers to any laboratory-controlled stress without prejudgement on its intensity, duration, and frequency. But in most cases, an experimental stress corresponds to a stronger and (or) longer and (or) more frequent stress than encountered in the wild.

Biomarkers can be used as predictive tools when they are considered at the population or community scale. But linking individual records to the population response is the most difficult task, since this implies not only the development of the biomarker itself and knowledge of its baseline expression, but also further (usually experimental) studies to determine the effects at the population level. These effects can be diverse and affect the organism in several ways (sexual behaviour and mating, locomotion, feeding, cellular and tissular functions, or genetic alterations; see Lagadic 2002). Most of the time, these effects imply an alteration of the reproductive success, which constitutes the main relationship between the individual and population levels. The evidence of the relationship between these two biological levels has previously been successfully tested and is currently used to predict the effects of some pollutants on marine communities (see Lagadic 2002 for a review of these examples).

In the present work, we provide a background for the future use of HSPs as biomarkers of the biological and ecological effects of global warming on marine communities. Our work shows that the development and the generalization of the use of HSPs as biomarkers of the effects of global warming can be conducted rapidly and at a reasonable cost. In this perspective, their ubiquitous presence and quick response to any change of temperature make HSPs excellent candidates. The use of a Mediterranean (and non-model) species influenced by global warming and living in a poorly resilient habitat where the effects of warming can be extremely drastic (see Chevaldonné and Lejeusne 2003; Lejeusne and Chevaldonné 2005) is further evidence of the ecological relevance of HSPs. They may prove useful in a diagnostic approach of the fine-scale mechanisms leading from a thermal anomaly to a disease outbreak or a shift in distribution (Pérez et al. 2000; Chevaldonné and Lejeusne 2003). After further experimental and in situ studies, HSPs might also be useful in a predictive approach for evaluating the potential effects of the warming trends at higher biological levels of organization.

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Effects on periphyton and macroinvertebrates from removal of submerged wood in three Ontario lakes

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Abstract: We removed 40%–70% of nearshore wood habitat from three lakes to test the link between habitat and productive capacity, specifically focusing on the provision of substrate for periphyton and invertebrate production by submerged wood. Our objectives were fourfold: (*i*) to calculate the total amount of invertebrate and periphyton biomass removed with the wood; (*ii*) to explore wood's value as habitat for invertebrates and periphyton; (*iii*) to determine the response within residual epixylic periphyton and invertebrate biomass; and (*iv*) to assess interactions between periphyton and invertebrates and other factors that may influence wood's productivity. Invertebrate biomass was greater on wood than in sediment, but the total available sediment area exceeded that of wood, thus a relatively small proportion of overall productivity was lost. Highly decayed wood supported greater chlorophyll *a* concentrations and more invertebrate biomass and diversity than fresh wood. The removal had no measurable effect on whole-lake water chemistry, nor did it result in a response in residual epixylic periphyton and invertebrate biomass. We conclude that we permanently reduced a dynamic and concentrated biomass of primary and secondary productivity in lakes by removing submerged wood habitat.

Résumé : Nous avons retiré 40%–70% de l'habitat de bois près des rives de trois lacs afin de tester le lien entre cet habitat et la capacité de production, plus particulièrement la fourniture par le bois submergé d'un substrat pour la production du périphyton et des invertébrés. Nous avons quatre objectifs: (*i*) calculer la quantité totale de biomasse d'invertébrés et de périphyton retirée avec le bois; (*ii*) déterminer la valeur du bois comme habitat pour les invertébrés et le périphyton; (*iii*) connaître la réaction de la biomasse résiduelle de périphyton et d'invertébrés sur le bois et (*iv*) mesurer les interactions entre le périphyton et les invertébrés, ainsi que les autres facteurs susceptibles d'influencer la productivité du bois. La biomasse d'invertébrés est plus importante sur le bois que sur les sédiments; cependant, comme la surface totale de sédiments disponible est plus grande que celle du bois, une partie relativement petite de la productivité globale est perdue. Le bois très décomposé supporte une concentration de chlorophylle *a*, ainsi qu'une biomasse, une diversité et une richesse d'invertébrés plus élevées que le bois frais. Le retrait du bois reste sans effet mesurable sur la chimie de la masse d'eau totale du lac et il ne provoque pas de réaction dans la biomasse résiduelle de périphyton et d'invertébrés. En conclusion, le retrait dhabitat de bois submergé réduit de façon permanente une biomasse dynamique et localisée de productivité primaire et secondaire dans les lacs.

[Traduit par la Rédaction]

Introduction

The presence of wood in aquatic systems has long been considered beneficial by fisheries resource managers (e.g., Keast et al. 1978; Bassett 1994; Bolding et al. 2004). The structural complexity supplied by submerged wood provides protection from predation (Everett and Ruiz 1993; MacRae and Jackson 2001), particularly for smaller or juvenile fish (Moring et al. 1989; Quinn and Peterson 1996; Cederholm et al. 1997), and can decrease intraspecific competition through visual isolation (Sundbaum and Näslund 1998). Wood provides a surface for growth of periphyton (Lebkuecher et al. 1998) and can be important as a site of invertebrate production (Bowen et al. 1998). Many cyprinids consume periphyton as a main food item, and benthic invertebrates constitute an important component of fish diets (Hecky and Hesslein 1995;

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Table 1. Various lake parameters, total area of wood surface area available for production (SAP; W.G. Cole, unpublished data), emergent macrophytes (Frezza 2001), and open sediment (Frezza 2001) available in each lake before wood removal.

Lake	Lake perimeter (m)	Lake area (ha)	Mean depth (m)	Secchi depth (m)[a]	Wood SAP (m^2)[b]	Macrophytes (m^2)	Open sediment (m^2)
Lower Batchawana	1292	5.8	3.3	4.3 (0.10)	410	218	6 221
Little Turkey	2355	21.6	6.0	4.8 (0.14)	2089	2 666	20 033
Wishart	3011	19.2	2.2	3.1 (0.08)	1538	20 589	49 369
Quinn	1418	6.4	4.7	3.5 (0.10)	2792	182	8 851

[a]Mean (with standard error in parentheses) across years of study of each lake.
[b]SAP = surface area × (% submerged – % buried).

Schindler and Scheuerell 2002). While wood has been shown to contribute directly to fish production (Benke et al. 1985; Keshavanath et al. 2001) and influence fish growth (Schindler et al. 2000), the specific role of wood as a surface for production of potential forage for fish, particularly in lakes, has received little attention (Schindler and Scheuerell 2002), possibly because of the difficulty in sampling this highly heterogeneous habitat or because of the relatively low density of wood in frequently studied large lake ecosystems.

Previous research has demonstrated that some of the complex ecological interactions among periphyton, phytoplankton, and invertebrates are affected by resources such as habitat type, light, and nutrients (Vadeboncoeur et al. 2002). In a whole-lake experiment, Vadeboncoeur et al. (2001) found that periphyton on wood responded positively to fertilization, suggesting direct competition with phytoplankton for nutrients. The same study showed that area-specific periphyton production on wood was 5–10 times lower than on sediments and comprised no more than 4% of benthic primary production when extrapolated to the system level. In contrast, O'Connor (1991) found that for invertebrates, greater species richness occurred on complex woody habitat relative to simple habitat and adjacent sediment, hypothesizing that complex woody habitat provided more resources and better oxygen availability than benthic sediment. These contrasting results could be due to the significant negative effects invertebrates may have on periphyton biomass (Hillebrand 2002).

In Canada, the federal department of Fisheries and Oceans Canada (DFO) is responsible for enforcing the Fisheries Act. This act protects fish habitat from any activity that results in its harmful alteration, disruption, or destruction (HADD) through a policy of no net loss of the productive capacity of fish habitat, where productive capacity is defined as "the maximum natural capability of habitats to produce healthy fish, safe for human consumption, or to support or produce aquatic organisms upon which fish depend" (DFO 1986). In this context, submerged material such as wood that contributes to the food web, directly or indirectly, may be considered as part of the productive capacity of fish habitat. When a development activity results in a HADD, provisions are made to compensate for the loss by creating or enhancing habitat elsewhere (DFO 1986, 1998). One key and relatively untested assumption underlying such decisions is that habitat quantity and quality are directly related to productive capacity. However, the effects of habitat perturbation or the success of habitat enhancement is rarely assessed from a biological perspective, such as by measuring changes in biotic biomass or production resulting from designed changes in habitat (Smokorowski et al. 1998).

This paper focuses on one potential role of wood as fish habitat, namely the provision of substrate for periphyton and invertebrate production. Our experiment was designed as part of a framework of habitat manipulations intended to test hypotheses about lake productive capacity and fish community response to habitat change (Kelso et al. 2001). The chosen treatment was to remove wood from 50% of the nearshore areas of a suite of small (<25 ha) lakes to provide a coarse-scale empirical test of the relationship between fish habitat and productive capacity.

We will look beyond the simpler measure of square metres of habitat to the actual loss of epixylic (wood-associated) periphyton and invertebrates and the response of the system to the affected change. The objectives of this study were fourfold: (*i*) to calculate the total amount of invertebrate and periphyton biomass removed with the wood; (*ii*) to explore wood's value as habitat for invertebrates and periphyton; (*iii*) to determine the response within residual epixylic periphyton and invertebrate biomass; and (*iv*) to assess interactions between periphyton and invertebrates and other factors that may influence wood's productivity. Through this study, we examined how submerged wood contributes to the productive capacity of fish habitat in lakes.

Materials and methods

Sites

The experiment was conducted on three lakes in the Turkey Lakes Watershed (TLW) (Lower Batchawana (control), Wishart, and Little Turkey), located approximately 50 km north of Sault Ste. Marie, Ontario, Canada, (47°02′30″N, 84°24′30″W), and on Quinn Lake, located 17 km north of Sault Ste. Marie (46°43′52″N, 84°13′14″W). All four are undeveloped, oligotrophic lakes, protected from sportfishing and ranging in area from just under 6 ha to nearly 22 ha and in mean depth from 2 to 6 m (Table 1).

Habitat availability by type was quantified in each lake as part of a previous study (Frezza 2001), from which data for emergent macrophytes and open sediment were used to assess relative area potentially available for fish foraging in each lake. Estimates of the total volume and surface area of coarse wood available for production of potential forage for fish (SAP = surface area × (% submerged – % buried)) were calculated in each lake prior to the wood removal (W.G. Cole, unpublished data). Open sediment was by far the most

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available habitat in all lakes (Table 1). Prior to treatment, wood surface area exceeded aerial coverage of macrophyte habitat in Quinn and Lower Batchawana lakes; in Little Turkey, surface areas of wood and macrophytes were similar, while in Wishart Lake, macrophytes were more abundant (Table 1).

Wood removal

All vertical woody structure (course woody material plus attached fine woody material less than half buried or extending above the sediment >2.5 cm) in the nearshore area (from the high water mark to 2 m depth or 10 m from shore, whichever came first) was removed from 50% of the shoreline of Quinn, Little Turkey, and Wishart lakes. The length and location of removal areas were randomly selected, except for areas with active beaver lodges, which were excluded, resulting in cleared areas with contiguous lengths ranging from 140 to 506 m. The wood was removed manually using chainsaws and winches, then moved by a small barge to a landing where it was offloaded to a log truck for transfer to a nearby storage site. Wood was removed from Little Turkey Lake and Quinn Lake in autumn of 1999 and from Wishart Lake in autumn of 2000. Upper and Lower Batchawana had no wood removed; Wishart Lake also served as a control in July of 2000.

Field sampling

In each treatment lake, stratified random sampling was used to select five sampling sites from wood removal zones and at least five sites from undisturbed shoreline areas. In the control lake (Lower Batchawana), 10 sites were randomly selected. Sampling sites were approximately 20 m long. Samples were collected at the same time each summer in each of 3 years, from 1999 through 2001, except in Wishart Lake, which was sampled in 2000 and 2001 only.

At each site, three pieces of submerged wood (minimum 1 m length and 2 cm diameter somewhere along the length) were sampled at each annual visit. Each piece of wood was lifted from the water, and total length and circumference at three locations were measured, ensuring the sections to be removed for processing were not disturbed by the measurement. We classified wood decay as follows: Class 1, fresh, firm wood, bark intact; Class 2, bark loose or absent, surface slightly rotted; or Class 3, surface rotted, center solid or rotted (as modified from Bowen et al. 1998). The average surface area of wood sampled for periphyton chlorophyll *a* (range 42–44 cm^2 each) and invertebrate (range 244–299 cm^2 each) analyses was comparable among lakes. We attempted to select an even distribution among wood decay classes, but sometimes had difficulty finding enough new (Class 1) or very old (Class 3) samples (total across lakes and years: Class 1, $n = 93$; Class 2, $n = 250$; Class 3, $n = 205$).

All three wood pieces selected from each site were sampled for invertebrate analysis, while two of the three were sampled for chlorophyll *a*. For periphyton chlorophyll *a* analysis, a 3 cm section was cut and placed in a labeled plastic bag with 50 mL of tap water and three drops of $MgCO_3$ (a preservative). The bagged sample was placed inside an opaque bag, stored in a cooler on ice, and processed for chlorophyll *a* within 24 h. For invertebrate sampling, one section approximately 20 cm long was cut and placed in a labeled plastic bag with approximately 200 mL of filtered (253 µm) lake water. The sample was stored in a cooler on ice until processing within 24 h of field collection.

Supplemental variables measured within the suite of lakes included the following: (*i*) phytoplankton chlorophyll *a* and water chemistry (including nutrients, carbon compounds, major ions, pH, conductivity, and alkalinity) extracted from triplicate center-lake, epilimnetic, integrated samples collected monthly from April to October; (*ii*) benthic invertebrate biomass and diversity on open sediment in Little Turkey Lake from quantitative sweeps (six replicate 0.25 m^2 samples at 0.5 m depth in the littoral zone) collected in the summers of 2000 and 2001; and (*iii*) periphyton accumulation on artificial substrates (10 Plexiglas tiles, each 0.03 m^2, installed at a depth of 0.5 m for 8 weeks) deployed randomly in each lake each summer. The use of the artificial substrate was intended to provide an independent and relative measure of periphyton growth over a fixed period of time across lakes and years.

Lab processing

Periphyton

Each 3 cm wood section was cleaned with a soft toothbrush and tap water into a 4 L beaker, and the sample bag was rinsed into the beaker. The contents were transferred into a 500 mL beaker, and tap water was used to bring the volume to the nearest 50 mL. On a magnetic stirrer, 25 mL aliquots of the suspension were pipetted alternately onto each of two glass fiber filters (Whatman GF/C, 42.5 mm), continuing addition until the filtration slowed, then the volume filtered was recorded. The filters were sealed in plastic bags and frozen prior to chlorophyll *a* analysis (APHA 1985), resulting in a measure of biomass of chlorophyll *a* ($mg \cdot m^{-2}$). The cleaned wood was measured for circumference at three points and total length to calculate surface area (length × average circumference). The same extraction and analysis procedures were used for periphyton chlorophyll *a* sampling from the Plexiglas tiles.

Invertebrates

Each 20 cm wood section was cleaned with a soft toothbrush and tap water into a large plastic bucket; the sample bag was rinsed into the bucket. The contents of the bucket were filtered through a concentrating funnel (253 µm mesh) and preserved in 70% ethanol. Invertebrates were sorted from the accompanying debris with the aid of a dissecting microscope. Organisms were identified to the level of Order, enumerated, pooled and weighed to generate an estimate of biomass per unit area ($mg \cdot m^{-2}$) for each sample, and then averaged to generate mean values in each lake and year. The surface area of the sample was calculated as above, from the length and circumference of the cleaned wood.

Data analyses

Invertebrates and periphyton removed

Estimates of SAP were calculated separately for treated and untreated areas in each lake prior to the wood removal (W.G. Cole, unpublished data). Estimates for wood in the treated areas approximate the amount of wood removed.

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Table 2. Total volume and surface area available for production (SAP) of wood in each lake prior to removal and the volume, SAP, and percentages removed during treatment.

	Total wood		Wood removed			
Lake	Volume (m^3)	SAP (m^2)	Volume (m^3)	SAP (m^2)	Volume (%)	SAP (%)
Little Turkey	180	2089	107	1492	59	71
Quinn	163	2792	105	1779	64	64
Wishart	166	1538	66	622	40	40

Note: SAP = surface area × (% submerged – % buried).

Premanipulation mean values of amount (per unit of surface area of wood) of periphyton (biomass of periphytic chlorophyll *a*) and invertebrates (biomass) on wood were calculated for each lake. These values were multiplied by the estimated SAP removed from each lake to produce estimates of the total biomass of periphytic chlorophyll *a* and invertebrates removed with the wood, thus representing the amount of potential forage for fish lost from each lake because of the habitat perturbation.

Value of wood as habitat

Invertebrate biomass (biomass per unit area) on wood vs. open sediment in Little Turkey Lake was compared using a nested analysis of variance (ANOVA, substrate type(year)). The total biomass of invertebrates living on open sediments in Little Turkey Lake was estimated by multiplying invertebrate biomass per unit area from the quantitative sweeps with the surface area of open sediments from Frezza (2001); this was compared with invertebrates living on wood on a whole-lake scale. This comparison was made only in Little Turkey Lake, as this was the one lake sampled for invertebrates on open sediment, and only for post-treatment in 2000 and 2001.

We tested for differences in periphyton chlorophyll *a*, invertebrate biomass, and invertebrate diversity among wood decay classes using a one-way ANOVA across years and lakes. Two diversity indices were calculated for each invertebrate sample: richness (*S*) and the probability of interspecific encounter (PIE) (Hurlbert 1971). PIE is an unbiased evenness measure that measures the chance that two individuals drawn at random from a population represent different species:

$$(1) \qquad \text{PIE} = \sum_{i=1}^{s}\left(\frac{n_i}{n}\right)\left(\frac{n-n_i}{n-1}\right)$$

where n is the number of all individuals in the sample, n_i is the number of individuals of a species in the sample, and s is the number of species (Hurlbert 1971). PIE was selected over other diversity indices because it provides a statistically and biologically understandable probability, unlike more traditional diversity measures (Gottelli and Graves 1996). When significant ($p < 0.05$) effects were detected, Tukey's post hoc tests were used to determine which decay classes were different.

Before–after control–impact (BACI) model

The BACI experimental design has the potential to deliver scientifically defensible results when assessing the effects of an environmental disturbance (Underwood 1991, 1993; Minns et al. 1996). The design requires that the ecosystem components under investigation in the experimental system be measured before and after the impact occurs and that the same ecosystem components be measured in a similar but unmanipulated system during the same period. The BACI test determines if the impact system changed relative to the control system after the experimental perturbation.

Our staggered design, with multiple experimental sites treated in different years, necessitated the use of the more complex multiple BACI design (MBACI; Keough and Quinn 2000). The main factors of our model were treatment (Trt; i.e., wood removal or control), and before–after (Time), with sites (S) nested within Trt, and years (Y) nested within Time. The complete ANOVA model included the terms Trt, Time, Trt × Time, S(Trt), S(Trt) × Time, Y(Time), and Y(Time) × Trt. The key term is Trt × Time, tested using S(Trt) × Time mean square instead of the residual mean square, which measures any change associated with the wood removal (Keough and Quinn 2000). The assumptions of this design are that the control and impact sites are independent, the data display no serial correlation, and that the control and impact sites "track" well through time in the absence of the perturbation. All data were tested against a normal distribution (Kolmogorov–Smirnov test) and were log- or square-root-transformed if necessary to correct deviations. Data were also tested for serial independence (Sokal and Rohlf 1995) and were found not to violate that assumption.

Response to wood removal and trophic interactions

To examine the changes in the chemical environment in our study lakes due to the experimental treatment, water chemistry variables were placed into one of three groups: (*i*) major ions (Ca^{2+}, Mg^{2+}, Na^+, K^+, Cl^-); (*ii*) nonphosphorus nutrients (NH_4, SO_4, SiO_2, and total N); and (*iii*) acidity–hardness (pH, alkalinity, conductivity). For each group, a principal components analysis (PCA) was used to generate combined orthogonal variables, and the first principal component was then used in the MBACI analysis. Total P was analyzed separately, since its removal resulted in a greater percent variance explained in the nutrients PCA, and P alone is an indicator of lake trophic status.

The concentration of chlorophyll *a* in phytoplankton and epixylic periphyton and epixylic invertebrate biomass per unit area were plotted over time and then tested for a response to the wood removal using the MBACI model. To validate our periphyton results and to test the theory of competitive interaction between phytoplankton and periphyton, we used correlation analyses to test the relationship between chlorophyll *a* concentrations of epixylic periphyton vs. artificial substrates and between phytoplankton and epixylic periphyton, respectively. Invertebrate richness and PIE were plotted over time, and the effect of wood removal was tested

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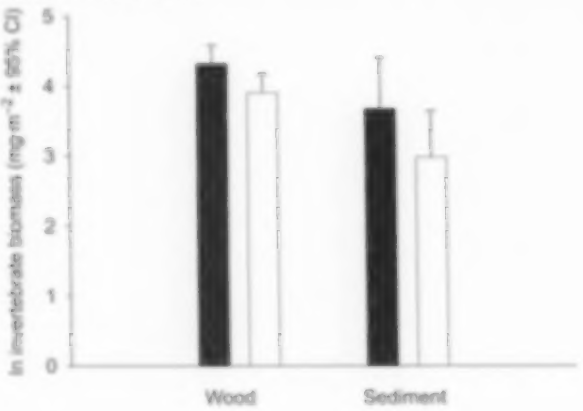
Fig. 1. Mean invertebrate biomass (ln + 1 transformed, ±95% confidence interval (CI)) on wood vs. open sediment in Little Turkey Lake in 2000 (solid bars) and 2001 (open bars). Substrate(year): $F_{(1,811)} = 5.4$, $p = 0.002$.



using the MBACI model. The trophic relationship between epixylic chlorophyll *a* and biomass of epixylic invertebrates was investigated using simple linear correlation analysis.

Results

Invertebrates and periphyton removed

The wood removal was designed to achieve 50% removal by shoreline perimeter distance, but because of the uneven distribution of wood along the shoreline, we removed between 40% and 70% of the total wood surface area available for production of invertebrates and periphyton (Table 2). At the time of the wood removal treatments, aerial biomass of chlorophyll *a* was 4.3, 23, and 22 mg·m^{-2} and invertebrate biomass was 59, 54, and 281 mg·m^{-2} in Quinn, Little Turkey, and Wishart lakes, respectively. Thus, as a result of the experimental treatment, a total of 8, 35, and 14 g of periphyton chlorophyll *a* and 105, 81, and 175 g (dry weight) of invertebrates were removed from Quinn, Little Turkey, and Wishart lakes, respectively.

Value of wood as habitat

Chlorophyll *a* biomass on the artificial substrates, which ranged from 0.4 ± 0.03 mg·m^{-2} (Quinn Lake, 2000) to 1.3 ± 0.18 mg·m^{-2} (Lower Batchawana, 2001, mean ± standard error) was less than the biomass on natural wood, which ranged between 4.3 ± 0.7 mg·m^{-2} (Quinn Lake, 1999) and 28.5 ± 3.7 mg·m^{-2} (Little Turkey, 2001), although this would be expected because of different roughness characteristics and colonization times between media. Invertebrate biomass (per unit area) was significantly greater on wood than in unperturbed sediment in Little Turkey Lake (Fig. 1). However, because of the disparity in total surface area between habitat types (Table 1), post-treatment in Little Turkey Lake, approximately 54 and 44 g of total invertebrate biomass remained on wood, and approximately 1005 and 438 g total invertebrate biomass was available in open sediment in 2000 and 2001, respectively.

The level of wood decay had a significant influence on both the quantity and quality of periphyton and invertebrates on the wood surface (Figs. 2*a*–2*d*). Post hoc analysis revealed that decay Class 3 wood supported significantly greater chlorophyll *a* concentrations, invertebrate biomass, invertebrate PIE, and invertebrate taxa richness than decay Class 1 wood ($p \leq 0.02$ in all cases; Figs. 2*a*–2*d*).

Response to wood removal and trophic interactions

Factor 1 scores from the three water chemistry principal components analyses accounted for a large proportion of variance among the raw variables (factor 1 eigenvalue percent variance: acidity–hardness 91%, major ions 63%, nutrients (no total phosphorus) 45%). Factor scores for water chemistry groupings showed no strong temporal pattern (Figs. 3*a*, 3*c*, 4*a*). Total phosphorus showed a slight decreasing trend in the TLW lakes, but not in Quinn Lake (Fig. 4*c*). We did not detect a significant effect on any of the water chemistry factors from the wood removal (Figs. 3*b*, 3*d*, 4*b*, 4*d*).

Temporal patterns in the concentration of chlorophyll *a* were similar in all four lakes, generally increasing on wood and decreasing in water across the 3 years (Figs. 5*a*, 5*c*). No significant changes as a result of the wood removal were detected in the concentration of phytoplankton or periphyton chlorophyll *a* on the remaining wood (Figs. 5*b*, 5*d*). There was a significant, positive correlation between epixylic periphyton and that on Plexiglas artificial substrates ($r^2 = 0.74$, $p < 0.001$) and a significant, negative correlation between concentrations of chlorophyll *a* in phytoplankton and periphyton ($r^2 = 0.58$, $p = 0.006$). The positive correlation between the wood and Plexiglas chlorophyll *a* provides an independent validation of our wood periphyton data and supports the idea that the snapshot of productivity found at the time of sampling is representative of current and recent conditions in the system.

High variability was apparent in invertebrate biomass among years, with both biomass and richness highest in 2000 in the TLW lakes (Figs. 6*a*, 6*e*). No impact on invertebrate biomass on wood was detected from the wood removal (Fig. 6*b*). Invertebrate diversity (richness and PIE) demonstrated no effect from the wood removal treatment (Figs. 6*c*–6*f*). We did not detect a relationship between mean periphyton chlorophyll *a* concentration and biomass of invertebrates (simple linear correlation, $r = 0.03$).

Discussion

Elimination of habitat

Resource management agencies and some literature (Christensen et al. 1996; Schindler et al. 2000; Jennings et al. 2003) have suggested that the simplification of nearshore structure in lakes, including removal of wood, is detrimental to aquatic communities. Our one-time, large-scale wood removal was considered to be a harmful destruction of the productive capacity of habitat by resource managers and scientists alike. In light of these prevailing beliefs, we were surprised when there was no apparent response of our measured variables to the wood removal. Yet, upon further consideration, we realized that it was specifically because of this non-result

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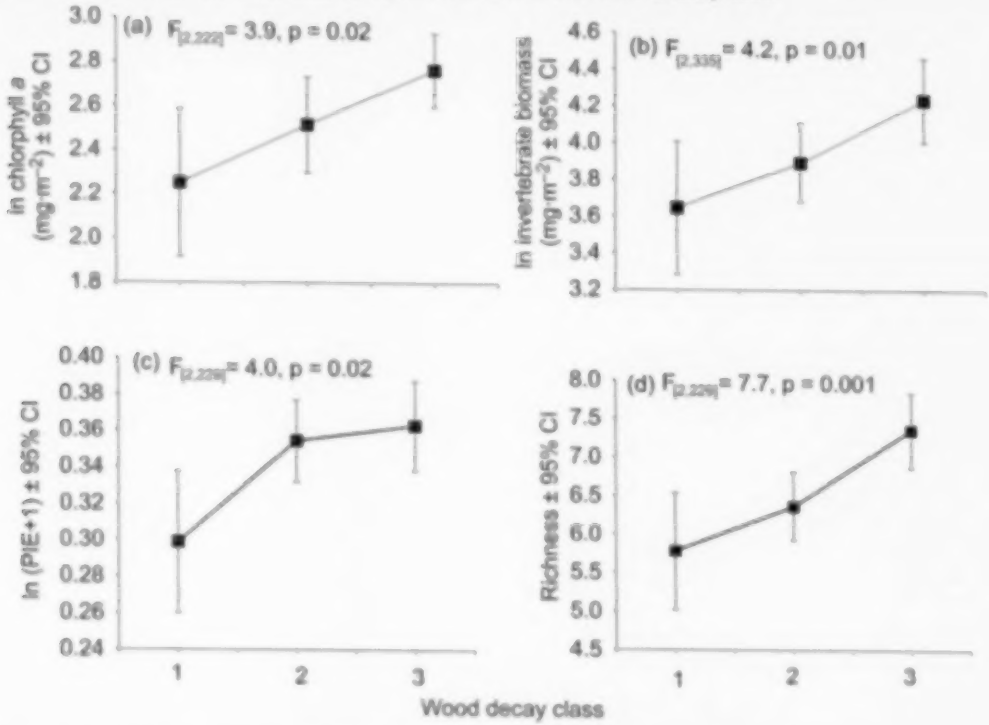
Fig. 2. Mean ± 95% confidence interval (CI) for (*a*) periphyton chlorophyll *a*, (*b*) invertebrate biomass, (*c*) invertebrate probability of interspecific encounter (PIE), and (*d*) invertebrate taxa richness at three levels of wood decay class.


(a) $F_{[2,222]}$ = 3.9, p = 0.02
ln chlorophyll *a* (mg·m^{-2}) ± 95% CI
(b) $F_{[2,335]}$ = 4.2, p = 0.01
ln invertebrate biomass (mg·m^{-2}) ± 95% CI
(c) $F_{[2,229]}$ = 4.0, p = 0.02
ln (PIE+1) ± 95% CI
(d) $F_{[2,229]}$ = 7.7, p = 0.001
Richness ± 95% CI
Wood decay class

that our findings should be of concern to resource managers. We have shown that the magnitude of wood removed and the loss of accompanying epixylic periphyton and invertebrate biomass in the absence of a response by residual communities should be considered harmful, since a large portion of a highly productive resource was eliminated.

Invertebrate biomass was significantly greater on wood than on open sediment, but with the area of open sediment habitat being an order of magnitude greater than that of wood habitat, the proportion of the whole-lake invertebrate community removed was small. Comparison of benthic habitat types on a whole-lake scale demonstrated that open sediment and associated productivity predominate. Unless a fish was restricted to foraging on woody habitat, and all other factors being equal, our large-scale removal effectively removed less than 10% of total invertebrate and periphyton biomass contributing to the productive capacity of habitat.

Yet, the contribution of woody habitat to lake ecosystems would be underestimated by allocating equal weight to different habitat types. Because of the lack of wood-specific research, support for perceived high value of wood is often made by considering wood as similar to macrophytes. Macrophytes are highly valued by managers, as they have been shown to add heterogeneity to habitat (Benson and Magnuson 1992), increase macroinvertebrate densities (Crowder and Cooper 1982), and contain diverse and complex fish communities relative to rocky or shallow mud (open) habitats (Pratt and Smokorowski 2003). Since the substrate of our study lakes is primarily bedrock overlain with a thin layer of organic material, vegetation is sparse (shallow Wishart Lake excepted), and we did not assess the relative contribution of macrophytes as habitat for invertebrates and periphyton. By area, woody habitat rivaled macrophytes in terms of availability, thus coarse wood is an important factor influencing habitat complexity and resource availability in our lakes for both invertebrates and fish.

The greater density of invertebrates on wood and its complex, three-dimensional physical structure may render it more accessible to fish as a substrate for forage production than open sediment. We could find no lentic system study with which to compare our results, but this is a similar finding to many stream studies that have found greater density or diversity of organisms on wood than on sand, organic sediments, or bedrock (e.g., Johnson and Kennedy 2003; Johnson et al. 2003; Phillips 2003). Benke et al. (1985) found that snags in a stretch of river represented only 4% of total habitat surfaces but supported 60% of total invertebrate biomass, and that four of eight major fish species obtained at least 60% of their prey biomass from snags.

Fish are often concentrated in more complex habitat areas relative to open sediment in lakes, including complex wood habitat relative to open habitat (Pratt 2004; Newbrey et al. 2005). In addition, from underwater video taken in some of the same lakes, fish in wood habitats made significantly more feeding attempts than fish observed in vegetated or open habitats, although the variability in feeding attempt data was high in all systems (Pratt et al. 2005). It is likely, therefore, that the removal of half the available wood surface

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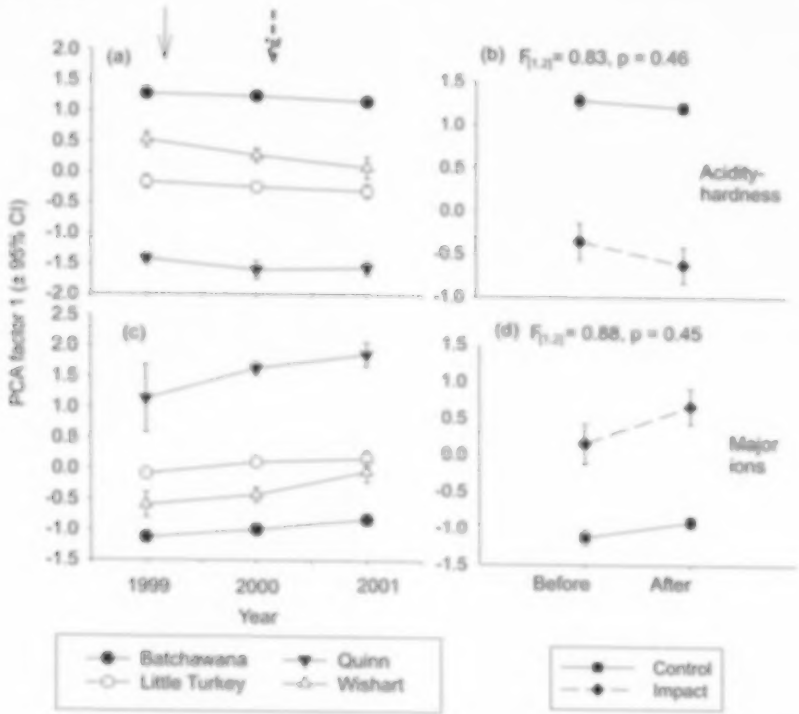
Fig. 3. Factor 1 scores from the principal components analysis (PCA) ±95% confidence interval (CI) for (*a*) acidity–hardness (pH, conductivity, alkalinity) in each lake over time; (*b*) acidity–hardness in the multiple before–after control–impact analysis of variance (MBACI ANOVA) model; (*c*) major ions (Ca^{2+}, Mg^{2+}, Na^+, K^+, Cl^-) in each lake over time; and (*d*) major ions in the MBACI ANOVA model. Solid arrow indicates when treatment occurred in Little Turkey and Quinn lakes; broken arrow indicates when treatment occurred in Wishart Lake.



area from a lake would reduce the energetic benefit of highly efficient feeding.

Value of wood as habitat

Chlorophyll *a* biomass on the artificial substrates was an order of magnitude less than the biomass on natural wood, but this would be expected because of different colonization times and surface roughness. In a similar study in two lakes in Algonquin Park, Ontario, Bowen et al. (1998) found that chlorophyll *a* on introduced wood substrates was almost three times less than natural wood, even after a 1-year colonization time. Mean chlorophyll *a* biomass on natural wood in the Algonquin lakes (23.3 ± 1.5 and 21.6 ± 1.9 mg·m^{-2}) was within the range of concentrations observed in our study. Thus, adding new wood to a system would not quickly replace periphyton lost with a natural wood removal.

In addition to providing a substrate for primary production (Vadeboncoeur and Lodge 2000) and being a direct food source for invertebrates (Hoffmann and Hering 2000; McKie and Cranston 2001), many physical characteristics of wood (e.g., hardness, surface structure, and decay class) may influence its suitability as substrate for invertebrates. Characteristics of fresh wood (bark intact, firm) differ from highly decayed wood (bark loose or absent, pitted) and may provide different taxa competitive advantages at various stages of decay. Bowen et al. (1998) found that invertebrate density and biomass was greatest on fresh, firm wood, with a community dominated by chironomids and mayflies. However, different patterns have been found in streams. Warmke and Hering (2000) saw no correlation between invertebrate abundance and wood density, surface conditions, or species. Magoulick (1998) found density of invertebrates was affected by the firmness and condition of wood, but not roughness, and Spänhoff et al. (2000) found density of invertebrates was significantly greater on conditioned vs. fresh wood, but was not dependent on genus. Most studies, regardless of habitat type, have found increasing diversity with increasing decay class, as the surface texture of the wood becomes more complex (e.g., O'Connor 1991; Collier and Halliday 2000). Our study also found greater chlorophyll *a*, invertebrate biomass, taxa richness, and PIE on more highly decayed wood.

Response to wood removal

The removal of the submerged wood in lake littoral zones had no measurable effect on whole-lake water chemistry, nor did it result in a response by residual epixylic periphyton and invertebrate biomass. Although a substantial proportion of wood surface area and accompanying epixylic productivity was removed from each lake, it is possible that the decrease in nutrient demand due to organisms removed was too small to induce a response in phytoplankton and epixylic periphyton. Alternatively, any nutrient benefit was negated by the loss of recyclable nutrients removed with the wood. Since all possible sources of periphyton and nutrient pools

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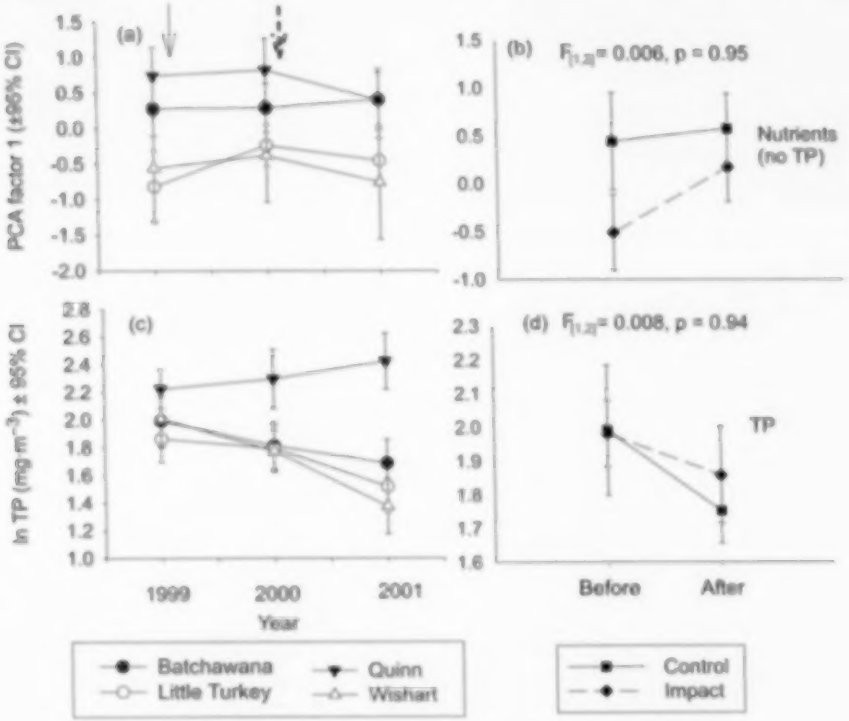
Fig. 4. Factor 1 scores from the principal components analysis (PCA) ±95% confidence interval (CI) for (*a*) nutrients (NH_4, SO_4, SiO_2, total Kjeldahl nitrogen, total nitrogen) in each lake over time; (*b*) nutrients in the multiple before–after control–impact analysis of variance (MBACI ANOVA) model; (*c*) total phosphorus (TP, ln + 1 transformed) in each lake over time; and, (*d*) TP in the MBACI ANOVA model. Solid arrow indicates when treatment occurred in Little Turkey and Quinn lakes; broken arrow indicates when treatment occurred in Wishart Lake.



in the lake should have been measured to draw firm conclusions, the magnitude of effort to conduct such a level of sampling to detect even a 50% change in benthic algal biomass was beyond our abilities within the current study (Kahlert et al. 2002).

With the apparent lack of change in epixylic biomass, invertebrates remaining on wood did not benefit from an increased forage base. The possibility that invertebrate biomass was at carrying capacity per unit of wood surface prior to commencement of our study is discounted by the large year-to-year changes we measured. In fact, the high year-to-year variability in invertebrate biomass may have contributed to our inability to detect a response in this group. It is also possible that a response occurred in the invertebrate community occupying newly available near-shore sediments, which was not measured in this study. Alternatively, if an increase in invertebrate biomass had occurred, it may have been masked by increased foraging pressure by fish concentrated in the less-available woody habitat. Because the wood was removed along sections of shoreline ranging up to 506 m in contiguous length, large areas exist along the shoreline where both forage for fish on wood and cover from wood are relatively scarce. Gaps in protective cover may restrict or limit invertebrate and fish dispersal by rendering them more vulnerable to predation and may alter community dynamics.

Trophic interactions

Periphytic and planktonic algae coexist in all lakes, but with similar nutrient and light requirements, these two forms of primary producers have dynamic competitive interactions, both spatially and temporally. This competition is evident in the negative relationship we found between planktonic and periphytic chlorophyll *a*. In general, phytoplankton by their free-floating nature have first access to light and can reduce light availability to periphyton by shading (Hansson 1988; Scheffer et al. 1993). However, periphyton has an advantage in its ability to sequester nutrients from the surface on which it grows (Schindler et al. 1987; Hagerthey and Kerfoot 1998). Vadeboncoeur and Lodge (2000) found that epixylic periphyton responded to fertilizer inputs to the ambient water column, whereas sediment-borne algae did not, suggesting that the primary nutrient source for the former is in the water column, but that of the latter is in the sediment. While competition with phytoplankton for nutrients may be greater for epixylic periphyton, a small increase in nutrients available due to periphyton removed (if it occurred) could be quickly taken up by either or both types of primary producers, an effect likely too small to detect over scales used in our study.

These limiting mechanisms are coupled with top-down control of primary production by secondary consumers, which

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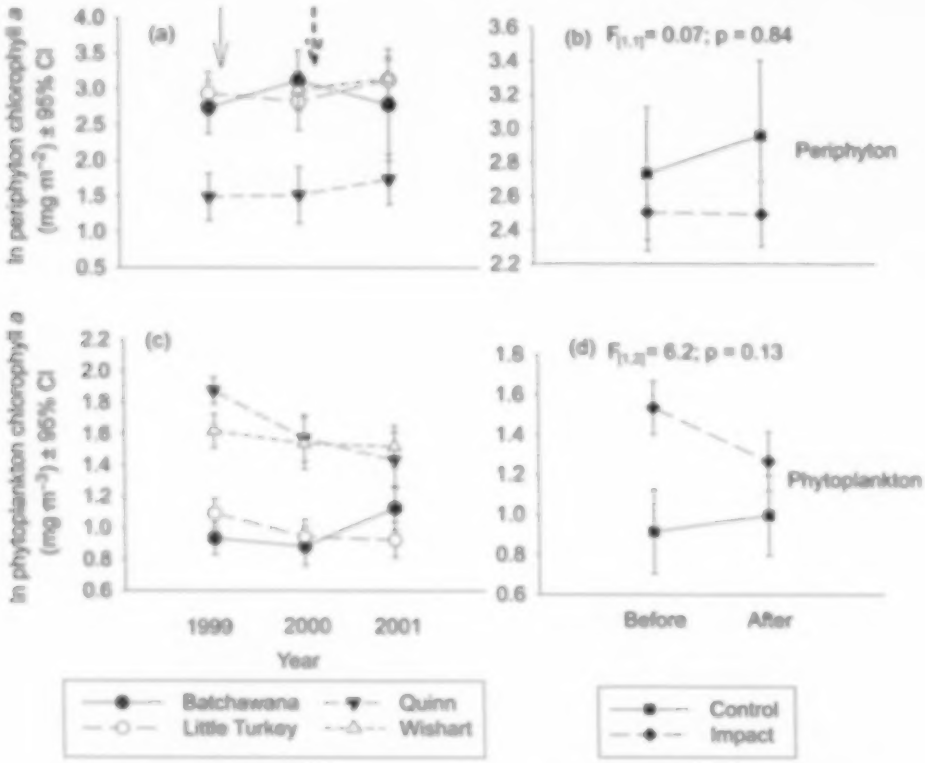
Fig. 5. Periphyton and phytoplankton chlorophyll *a* (ln + 1 transformed) ±95% confidence interval (CI) for each lake over time (*a* and *c*, respectively) and in the multiple before–after control–impact analysis of variance (MBACI ANOVA) model (*b* and *d*, respectively). Solid arrow indicates when treatment occurred in Little Turkey and Quinn lakes; broken arrow indicates when treatment occurred in Wishart Lake.



can have a major effect by removing and shaping periphyton communities, sometimes to a greater extent than nutrients and light (Hillebrand 2002). Numerous feedback mechanisms create complex relationships between the benthic community and the periphytic communities. Hillebrand et al. (2002) found that macroinvertebrates selectively grazed algae from biofilm, tempering the positive effects of increased nutrients. As well, macroinvertebrates had positive, indirect effects on heterotrophic components of the periphyton assemblage, possibly via influences on nutrient supply and the algal size structure (Hillebrand et al. 2002). Neither our study nor Bowen et al. (1998) detected a significant relationship between chlorophyll *a* and biomass of invertebrates, possibly as a result of complex interactions.

Habitat management implications

The overall importance of benthic resources to lake ecosystems, the importance of benthic consumption to pelagic productivity, and the true complexity of nutrient and energy pathways in lake ecosystems has recently been recognized (Schindler and Scheuerell 2002; VanderZanden and Vadeboncoeur 2002). Hecky and Hesslein (1995) used stable isotope analysis to determine that diverse top consumer fish assemblages consumed equal proportions of benthic and planktonic algal carbons despite the very small estimated percent contribution of benthos to carbon production. The study's authors hypothesized that the energy efficiency in harvesting relatively concentrated benthic algae diet, plus the fact that benthic algae (being carbon-limited) may be more nutritious than phytoplankton (generally phosphorus- or nitrogen-deficient), was driving the preferential diet selection.

In many small, undeveloped lakes in forested watersheds, large accumulations of submerged wood constitute a large portion of allochthonous material and physical habitat complexity (Christensen et al. 1996). These large deposits of wood could support an important portion of primary and secondary production in such lakes and could take centuries to naturally reaccumulate once removed (Guyette and Cole 1999). Lake residential development has the effect of substantialy reducing coarse woody debris in littoral zones (Christensen et al. 1996), which has been linked to reduced fish growth and a corresponding reduction in the capacity of lakes to support productive fish populations (Schindler et al. 2000). While the importance of benthic energy pathways to lake ecosystem dynamics has been acknowledged and examined, there is still a surprising paucity of research evaluating the role of wood as benthic habitat in lakes, which is a major knowledge gap that should be filled. Resource managers should continue to consider wood as valuable habitat, since it provides a highly concentrated and accessible source of periphyton and invertebrate biomass, and unlike macrophytes, it will not naturally be restored in the short term. Even with

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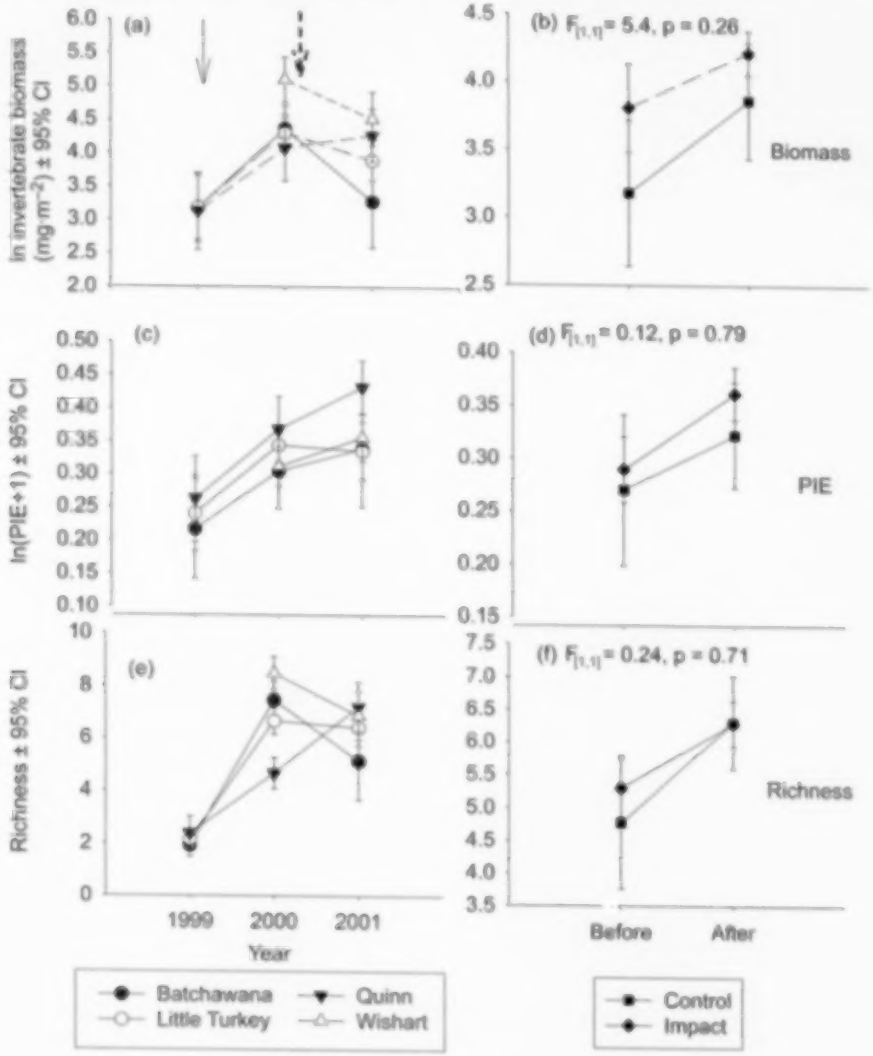
Fig. 6. Invertebrate biomass (ln + 1 transformed), probability of interspecific encounter (PIE, ln + 1 transformed), and taxa richness ±95% confidence interval (CI) for each lake over time (*a*, *c*, *e*, respectively) and in the multiple before–after control–impact analysis of variance (MBACI ANOVA) model (*b*, *d*, *f*, respectively). Solid arrow indicates when treatment occurred in Little Turkey and Quinn lakes; broken arrow indicates when treatment occurred in Wishart Lake.



human-assisted restoration of wood in aquatic systems, it may be years before productivity and diversity are maximized as decay progresses.

In conclusion, the removal of a large proportion of wood from lake littoral zones did not induce a detectable change in water chemistry, phytoplankton standing crop, epixylic periphyton standing crop, or invertebrate biomass on the remaining wood. From a system-wide perspective, it appears that the large-scale habitat destruction did not induce short-term response in these communities. From the fishes' perspective, however, treated areas in these lakes would no longer be as productive to inhabit as areas with abundant wood. We demonstrated that wood provides a dynamic and concentrated biomass of primary and secondary productivity relative to other benthic forage bases, and its role in lakes warrants further exploration.

Acknowledgements

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Characterization of elasticity patterns of North American freshwater fishes

Luis A. Vélez-Espino, Michael G. Fox, and Robert L. McLaughlin

Abstract: We applied elasticity analysis to 88 North American freshwater fishes to assess the relative impacts of changes in the vital rates on asymptotic population growth. Variance in vital rates was summarized for four distinct functional groups: (*i*) species with population growth rates strongly sensitive to perturbations in adult survival; (*ii*) species with population growth rates sensitive to perturbations in overall survival; (*iii*) species with population growth rates most sensitive to perturbations in juvenile survival; and (*iv*) species with population growth rates sensitive to perturbations in juvenile survival and fecundity. The results of the present study also showed that (*a*) elasticity patterns cannot be inferred in a straightforward manner from trade-offs between life-history traits, (*b*) the sensitivity of a population's growth rate to changes in adult survival and fecundity can be predicted empirically from life span and age at maturity, respectively, (*c*) elasticities are highly conserved among genera within the same taxonomic family, and (*d*) there are key divergences between elasticity patterns of freshwater fish and other vertebrate taxa.

Résumé : Nous avons soumis à une analyse d'élasticité 88 poissons d'eau douce d'Amérique du Nord afin d'évaluer les impacts relatifs des changements de leurs taux vitaux sur leur croissance asymptotique de population. Nous avons résumé la variance des taux vitaux pour quatre groupes fonctionnels distincts : (*i*) les espèces dont le taux de croissance démographique est sensible aux perturbations dans la survie des adultes, (*ii*) les espèces dont le taux de croissance démographique est sensible aux perturbations dans la survie globale, (*iii*) les espèces dont le taux de croissance démographique est le plus sensible aux perturbations dans la survie des jeunes et (*iv*) les espèces dont le taux de croissance démographique est sensible aux perturbations dans la survie des jeunes et dans la fécondité. Les résultats de notre étude montrent que (*a*) on ne peut déduire les patrons d'élasticité de manière directe des compromis entre les caractéristiques du cycle biologique, (*b*) on peut prédire de manière empirique la sensibilité du taux de croissance démographique aux changements de survie des adultes et de fécondité, respectivement à partir de la durée de vie et de l'âge à la maturité, (*c*) il y a une forte conservation des élasticités parmi les genres au sein d'une même famille taxonomique et (*d*) il y a des différences fondamentales entre les patrons d'élasticité des poissons et ceux des autres taxons de vertébrés.

[Traduit par la Rédaction]

Introduction

A central goal of ecology is to predict population dynamics from demographic data (Saether and Bakke 2000). To date, an essential question of life-history theory is how variation in demographic traits affects fitness (Brommer 2000). However, few comparative analyses examined how life-history features affect demographic traits and, ultimately, population growth rate. A collective effort has searched for general effects that variation in a demographic trait has on the population growth rates across several taxa, finding that the relative contribution to population growth rate by different stages in the life cycle may differ significantly among taxa (Heppell et al. 2000; Saether and Bakke 2000; Cortés 2002). At the same time, there is growing need for a broad-scale summarization of species' population dynamics that could help conservation ecologists and resource managers anticipate how individual species might respond to human alterations of the environment. This information is needed to help conserve biodiversity and ecosystem function in aquatic systems that have not been altered appreciably by human activities, as well as to help restore systems that have been heavily influenced by human activities (Caughley and Gunn 1996).

Such a summarization is needed for freshwater fishes in particular because (*i*) extinction rates estimated for aquatic organisms are higher than for terrestrial organisms, (*ii*) North American freshwater biodiversity is diminishing at an alarming rate (Ricciardi and Rasmussen 1999), with more than 20% of North America freshwater fishes listed as imperiled (Williams et al. 1989) and 88% of fish species listed as threatened or endangered by the US Fish and Wildlife Service corresponding to freshwater fishes (Winemiller 2005), and (*iii*) for most fishes, particularly those of limited economic and recreational significance, the information regarding life histories and population dynamics is limited. A summarization that identifies points in a species' life history that are sensitive to perturbations would be valuable for predicting species' vulnerabilities before any major decline, for

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identifying potential causes of declines, and for facilitating actions to reverse the decline. In addition, such comparative analyses among freshwater fish would contribute important information to the search for general patterns linking life history, demography, and population growth rate.

In this paper, we conducted a comparative analysis of how perturbations of demographic traits affect the population dynamics and fitness of North American freshwater fishes. Our comparison is based on elasticity analysis, a mathematical tool assessing the relative effects of proportional changes in the survival, growth, and fecundity vital rates on asymptotic population growth rate (De Kroon et al. 2000). Elasticity analysis can be used to identify the most vulnerable ages or stages in a life cycle, thereby providing a useful guide for research and management of species with poorly understood biology (Heppell et al. 2000). Interspecific comparisons based on elasticity analyses have been conducted on plants (Silvertown et al. 1993), birds (Saether and Bakke 2000), turtles (Cunnington and Brooks 1996), sharks (Cortés 2002), and mammals (Heppell et al. 2000). We limited our study on freshwater fishes because they have a more restricted range of life-history strategies than marine fishes (Winemiller and Rose 1992) and because life-history traits (especially recruitment) of freshwater fishes are apparently less variable than those of marine fishes (Powles et al. 2000; Hutchings 2001). Earlier reports have sought to classify freshwater fishes on the basis of their life histories without explicit use of the life-history analysis to infer vulnerability or sensitivity to perturbations in the vital rates (e.g., Wootton 1984; Winemiller and Rose 1992). Winemiller and Rose (1992) used correlations among demographic variables to infer life-history strategies of fishes and classify species based on their life history. Using these life-history summaries to make management decisions remains a pressing challenge. Rose (2005) analyzed the responses to changes in vital rates of 10 fish species using matrix and individual-based models without yielding consistent patterns in life-history space. It has been shown that a negative correlation between elasticities implies an exchange of contributions to fitness between two traits but does not imply an identical pattern of correlation between the same traits (see De Kroon et al. 1987). In addition, relationships between elasticities of different vital rates explain fitness consequences that cannot be determined from trade-offs between the values of the life-history traits.

Our comparative elasticity analysis entailed four steps. First, we estimated elasticities for juvenile survival, adult survival, and fecundity for 88 North American freshwater fishes using life-history data from the literature. This step was conducted to explicitly link life-history traits to attributes of population dynamics that could be used to make conservation or management decisions. Second, we classified species into functional groups that exhibited similar elasticity patterns and, hence, would be expected to exhibit similar responses to the same environmental perturbation. Third, we examined how trade-offs between life-history traits relate to (*a*) trade-offs among elasticities and (*b*) relationships between traits and elasticities, and we identified life-history predictors of the elasticity estimates. Steps 2 and 3 were conducted to facilitate inferences about population dynamics as new life-history data become available, given the limited data and that the available data for a given species may not be representative of every population. Fourth, we tested whether species of the same taxa (i.e., genus, family, order) exhibited similar elasticity estimates. If elasticities are highly conserved within taxonomic groups, then the population dynamics of species lacking suitable information could be inferred from the dynamics of a close relative.

Materials and methods

Data sources

Elasticity analysis required compilation of life-history data from the literature, development of a general matrix model suitable for all of the species, estimation of fecundity rates and survival probabilities (the matrix elements), and estimation of elasticities for each species using the matrix model. We initially obtained estimates of age at maturity, length at maturity, longevity, clutch size, and number of reproductive batches for 101 freshwater species from Winemiller and Rose (1992). The individual trait values are averages for populations located at the center of their North American geographical range. We augmented these data with information obtained from Fishbase (www.fishbase.org), Tennessee Animal Biogeographic System (http://fwie.fw.vt.edu/TN), and Scott and Crossman (1973). If more than one trait value was available per species, we selected the mean for continuous data (e.g., clutch size) and the median for discrete data (e.g., age at maturity). Gaps in our table of life-history traits, representing 1.2% of the total number of entries, were filled with estimates of the genus average for the trait in question. We then constructed individual life tables and stage-structured projection matrices for the 101 species.

The matrix model

Our population dynamics model used stage-structured projection matrices, which project how a hypothetical population of a species would behave assuming that the age distribution within stages was stable and the environmental conditions were maintained for an extended period of time (see Lefkovitch 1965). Age-structured matrices are a special case of the more general stage-structured matrices. The former divide the population into equal age classes, and in the latter, the fundamental assumption is that all individuals in a given stage are subject to identical mortality, growth, and fecundity schedules (Crouse et al. 1987). In those cases in which age-specific information is available, an age-structured model could be a better option, but even there the implementation of meaningful stages can be equally efficient. For our study, we did not count with complete life tables, and constructing and manipulating matrices of large dimensions (e.g., 80 × 80 in the case of lake sturgeon) for such a large number of species is time consuming and unnecessary for the purposes of this comparative study. A stage-structured approach requires partitioning the life cycle at biologically meaningful points and uses estimates of fecundity and survival specific to each stage. For each species, the projection matrix was constructed for a projection interval of 1 year and was based on five life stages: young-of-the-year (YOY), early juvenile, late juvenile, early adult, and late adult (Fig. 1). In our model, the juvenile stage is the period from the end of the first year to the age at maturity, with the early and late juvenile stages being the first and second half of this period,

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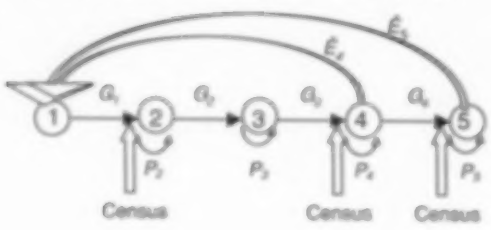
Fig. 1. (*a*) Generalized life cycle, (*b*) corresponding stage-structured projection matrix, and (*c*) matrix including formulas applied to calculate corresponding matrix elements used to model the population dynamics of North American freshwater fishes. The life cycle was divided into five stages: (1) young-of-the-year, (2) small juveniles, (3) large juveniles, (4) young adults, and (5) old adults. $\hat{E}_j$, the stage-specific fecundity rate; P_j, the probability of surviving and remaining in the same stage; G_j, the probability of surviving and moving to the next stage. The annual survival probability of an individual in stage j is σ_j, and the probability of growth from j to $j + 1$ given σ_j is γ_j.

(a)



(b)

$$A = \begin{pmatrix} P_2 & 0 & \hat{E}_4 & \hat{E}_5 \\ G_2 & P_3 & 0 & 0 \\ 0 & G_3 & P_4 & 0 \\ 0 & 0 & G_4 & P_5 \end{pmatrix}$$

(c)

$$A = \begin{pmatrix} \sigma_2(1-\gamma_2) & 0 & F_4\sigma_1 & F_5\sigma_1 \\ \sigma_2\gamma_2 & \sigma_3(1-\gamma_3) & 0 & 0 \\ 0 & \sigma_3\gamma_3 & \sigma_4(1-\gamma_4) & 0 \\ 0 & 0 & \sigma_4\gamma_4 & \sigma_5(1-\gamma_5) \end{pmatrix}$$

respectively. The early and late adult stages refer to the first and second half of the adult period, respectively, which covers the period from first reproduction to maximum observed age at reproduction. The subdivision in early and late juveniles and early and late adults was applied to reduce the loss of information caused by averaging vital rate values with high variance in species with long juvenile and (or) adult periods (see Crouse et al. 1987).

We used a birth-pulse model without overlapping generations to accommodate the seasonal reproduction exhibited by North American freshwater fishes. We used a prebreeding form of birth-pulse model, which assumes that demographic data are measured before the reproductive season. Formulas for survival and fecundity differ between pre- and post-breeding models and depend on when the census of the hypothetical population is assumed to occur.

Estimating fecundity rates and survival probabilities

Fecundity (F) was estimated as the annual production of female offspring per adult female, assuming 50% of offspring were female (i.e., sex ratio of 1:1) as

(1) $F = (\text{clutch size} \times \text{annual number of clutches})/2$

A linear regression was used to estimate age-specific fecundity (F_j) because the relationship between age and fecundity is typically linear within a fish species (Bagenal 1978). Our regression was generated by relating values of minimum and maximum fecundity with age at maturity and oldest age of reproduction, respectively. Fecundity rate (E_j) was estimated as the product of age-0 survival (S_0) and fecundity (F_j), and mean stage-specific fecundity rates ($\hat{E}_j$) of mature individuals were estimated from the arithmetic mean of within-stage-specific fecundities (E_j).

Survival probabilities were estimated from life-history invariants (see Charnov 1993) because field measurements of survival for most fishes are rare (see Cortés 2002). Adult survival (S_A) was assumed to be constant across early and late adult stages and was estimated as the arithmetic mean value from two empirical equations (Hoening 1983; Jensen 1996), incorporating into a single estimate the empirical relationships between mortality and both age at maturity and longevity. Jensen's equation estimates mortality as $M = 1.65/\alpha$, where α is the age at maturity. Hoening's equation estimates mortality rate as $M = e^{1.46 - 1.01 \ln(t_{max})}$, where t_{max} is maximum life span (i.e., longevity). When Jensen's equation was used, species with $\alpha = 1$ were assigned an adult mortality of 0.95. Hoening's method provides consistent results for $t_{max} \geq 5$, the range of ages considered in Hoening's model (see Hoening 1983); mortality rates for species with longevities of 4 years or less were calculated from the slope between the point defined by $t_{max} = 5$ and $S_A \mid t_{max} = 5$ and a fixed intercept of zero. Age-specific survival probabilities (S_i) for ages 1 to $\alpha - 1$ were estimated from adult survival as

(2) $S_i = (S_A)^i/\alpha$

where i represents the age class. This equation assumes a linear decrease in mortality with increasing length (Cushing 1974; Peterson and Wroblewski 1984) and was considered reasonable given the linear relationships between juvenile age and length described by von Bertalanffy (1957), Beverton and Holt (1957), and Pauly (1980).

As with all stage-structured models, it was necessary to separate the processes of survival and growth from one stage to another (see Brault and Caswell 1993). To do this, the survival probability of an individual in stage j (σ_j) and the probability of growth from j to $j + 1$ given σ_j (γ_j) were used to compute the probability of surviving and remaining in the same stage ($P_j = \sigma_j(1 - \lambda_j)$) and the probability of surviving and moving to the next stage ($G_j = \sigma_j \gamma_j$; the transition probability). The term γ_j was calculated from a geometric distribution as $\gamma_j = T_j^{-1}$, where T_j is the duration (years) of stage j. The term σ_j was calculated as the average within-stage age-specific survival probability, S_i. Stage 1 represents YOY. Therefore, $\sigma_1 = S_0$ and $P_1 = 0$ because the length of stage 1 is equal to the projection interval.

Juvenile survivorship from zygote until first reproduction was calculated as the product of the survival probabilities for YOY and juvenile stages (Fig. 1):

(3) $l_j = \prod_{j=1}^{3} (P_j + G_j)$

YOY survival was calculated by solving for S_0 under the assumption that the intrinsic rate of increase for the population

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(r) was 0. This involved a process of trial and error and a refinement of the estimate using elasticities (see next section).

Elasticity analysis

Annual population growth rate was estimated for each species from the maximum eigenvalue (λ) of each projection matrix. The parameter λ is directly related to the intrinsic rate of increase r ($\lambda = e^r$). The population is growing when $\lambda > 1$, at equilibrium when $\lambda = 1$, and declining when $\lambda < 1$. Elasticity analysis requires calculating the right and left eigenvectors derived for the projection matrix. Elements of the right eigenvector (w) represent the proportion of the population in stage class j given a stable stage distribution (McDonald and Caswell 1993). Elements of the left eigenvector (v) represent the number of progeny that an individual in stage class j is expected to produce during its entire life (stage-specific reproductive value; see Burgman et al. 1993). These eigenvectors are the mathematical operators for calculating the sensitivity of λ (Sen_{ij}), which measures the response of λ to perturbations in matrix elements a_{ij} (all the elements in Fig. 1). The sensitivity matrix is calculated as

$$\mathrm{Sen}_{ij} = \partial\lambda/\partial a_{ij} = (v_i,\ w_j)/\langle w,v\rangle \quad (4)$$

where $\langle w,v\rangle$ is the scalar product of the eigenvectors. The stage-specific survival and fecundity are measured on different scales and their values have different numeric ranges (0–1 for survival vs. 0 to F_i for fecundity), so λ can appear orders of magnitude less sensitive to changes in fecundity than to changes in survival (De Kroon et al. 1986). Elasticity (ε_{ij}) measures sensitivity on a proportional scale and is calculated as

$$\varepsilon_{ij} = (a_{ij}/\lambda)(\partial\lambda/\partial a_{ij}) \quad (5)$$

Elasticity also quantifies the relative contribution of vital rates to population growth rate (λ), and elasticities of the matrix elements sum to unity. The parameters λ, $\partial\lambda/\partial a_{ij}$, and ε_{ij} were calculated using MATLAB (version 6.5.1; The Mathworks, Inc. 2001).

The parameter λ becomes essential for estimating YOY survival, which was estimated initially by solving for $\lambda = 1$ and varying S_0 (and consequently E_1) while keeping all other matrix elements fixed, until the value of λ was within the range 0.9–1.1. The final refinement of S_0 was made using Caswell's (2001) formula to calculate the necessary proportional change in vital rates required to produce a specific change in λ:

$$\delta_{S_0} = \varepsilon_{S_0}^{-1}[(1-\lambda)/\lambda] \quad (6)$$

where λ is the population growth rate estimated by the trial and error estimate of S_0, δ_{S_0} is the necessary change in S_0 to produce equilibrium (i.e., $\lambda = 1$), and ε_{S_0} is the elasticity of S_0. Equation 3 is more accurate when values of λ are closer to 1 (see Caswell 2001), which necessitated the initial step of using iteration to obtain preliminary estimates of S_0.

The final estimate of YOY survival at equilibrium (S_0^*) was then calculated from δ_{S_0} as

$$S_0^* = S_0(1 + \delta_{S_0}) \quad (7)$$

where S_0 is the estimate generated through iteration. This step also standardized the individual matrix models' responses to changes in fecundity and survival rates that could be dependent on the state of the population. Declining, growing, or near to carrying capacity populations would likely show different responses to perturbations (Rose 2005).

Life histories lacking one or more of the life stages in the general life cycle (e.g., species maturing at age 1 do not have early and late juvenile stages as defined here; species maturing at age i with longevity $i + 1$ have only one adult stage) cause differences in matrix dimensionality. This was addressed following the standard approach of defining vital rate compartments common to all species (see Silvertown et al. 1993; Heppell et al. 2000). In animals, these compartments are usually juvenile survival, adult survival, and fecundity. Thus, the relative contributions of these composite vital rates were calculated using the additive property of elasticities as follows:

$$\text{elasticity of juvenile survival} = \varepsilon(S_J) = \varepsilon G_1 + \varepsilon P_2 + \varepsilon G_2 + \varepsilon G_3 + \varepsilon P_3 \quad (8)$$

$$\text{elasticity of adult survival} = \varepsilon(S_A) = \varepsilon G_4 + \varepsilon P_4 + \varepsilon P_5 \quad (9)$$

$$\text{elasticity of fecundity} = \varepsilon(F) = \varepsilon \hat{E}_4 + \varepsilon \hat{E}_5 \quad (10)$$

where subscripts represent each of the five stages of the general life cycle.

Clustering species based on elasticity patterns

Of the 101 species considered, 88 species belonging to 50 genera, 17 families, and 10 orders were used in following analyses. Thirteen species (*Campostoma anomalum*, *Nocomis biguttatus*, *Rhinichthys cataractae*, *Noturus gyrinus*, *Noturus miurus*, *Noturus alabaster*, *Noturus nocturnus*, *Aphredoderus sayanus*, *Percopsis omiscomaycus*, *Etheostoma zonale*, *Etheostoma variatum*, *Fundulus zebrinus*, and *Ammocrypta beani*) were not included in this analysis because they exhibited values of S_0 greater than juvenile or adult survival estimates, raising concerns that data available for these species were inaccurate. Variances in elasticities were categorized into groups with a weighted pair-group average (WPGA) clustering method using Euclidean distances determined from juvenile survival elasticity ($\varepsilon(S_J)$), adult survival elasticity ($\varepsilon(S_A)$), and fecundity elasticity ($\varepsilon(F)$). The WPGA method of clustering was selected because a three-dimensional (3-D) plot of elasticities suggested uneven cluster sizes, and weighting by cluster size increased the resolution of clusters (see Sneath and Sokal 1973). The number of clusters (p) and species memberships defined by the WPGA were corroborated with a second cluster analysis using a k-means clustering algorithm, which produces k different clusters of greatest possible distinction. The number of clusters in the k-means method was fixed at the number of distinct clusters discernable in the 3-D plot for WPGA. The greatest consensus (similarity expressed as percentage), with a minimum value of 95% between both cluster analyses, was used as a measure of significance for the appropriate number of clusters after varying the number of clusters to $p + 1$ and $p - 1$ and comparing the corresponding consensus percentages. The final ordination of elasticity clusters used a triangular plot (Silvertown et al.

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1993; Heppell et al. 2000), in which individual-axis values correspond to 90° extrapolations.

Mann–Whitney *U* tests were used to determine how elasticities of juvenile survival, adult survival, and fecundity differed among clusters. They were also used to determine whether age at maturity, reproductive life span, fecundity, juvenile survivorship, and longevity differed among clusters. Levels of significance were adjusted using a Bonferroni correction for simultaneous comparisons.

In addition, we qualitatively evaluated the correspondence between our elasticity clusters and the life-history strategies determined by Winemiller and Rose (1992). Winemiller and Rose (1992) classified North American fishes on the basis of demographic parameters such as juvenile survivorship, mean fecundity, and age at maturity (their fig. 6) and produced three life-history strategies termed "periodic" (high fecundity, late maturity, and low juvenile survivorship), "opportunistic" (low fecundity, early maturity, and low juvenile survivorship), and "equilibrium" (low fecundity, late maturity, and high juvenile survivorship). We evaluated the correspondence between elasticity clusters and Winemiller and Rose's life-history strategies by plotting the distribution of elasticity patterns, as indicated by clusters, within a triangular surface determined by the traits juvenile survivorship (l_1), age at maturity (α), and mean fecundity across the entire adult period (F). Trait values where standardized using a 0–1 scale before ordination in the triangular plot.

Life-history correlates of elasticity

We calculated Spearman rank correlations, adjusting levels of significance of $P < 0.05$ after a Bonferroni correction for simultaneous correlations, to examine how individual life-history traits were related to elasticities. Thereafter, we generated empirical linear regression equations with the greatest fit (i.e., greatest coefficient of determination, $P < 0.05$) for those trait–elasticity relationships characterized by strong and highly significant correlations.

Taxonomic affiliations

We used separate nested analyses of variance to estimate the proportion of total variation in elasticity accounted for by juvenile survival, adult survival, and fecundity that could be attributed to differences in taxonomic family within order, genus within family, and species within genus. We followed the allometry method described by Pagel and Harvey (1988), calculating the variance of species nested within genera from the species' mean. Genus means, based on the species' values, were used to calculate the variance of genera nested within family, and family means, based on the genera means, were used to calculate the variance of families nested within order. Higher order components were not considered because all orders were from the same taxonomic class, Actinopterygii. Elasticity values were arcsine square root transformed before analysis to normalize the distribution of values as closely as possible and meet the assumptions of parametric analysis of variance.

The dependence of elasticity clusters on taxonomic affiliations was tested with a $p \times q$ contingency table, where p represents the number of identified elasticity clusters and q indicates the number of taxa (e.g., order) with more than one subtaxa (e.g., families) within the taxonomic level identified by the allometry method as the most appropriate.

Fig. 2. Triangular plot depicting elasticities of juvenile survival, adult survival, and fecundity for 88 North American freshwater fishes. Each point represents a species. The type of point indicates the species' cluster affiliation: plus sign, cluster 1; solid diamonds, cluster 2; open squares, cluster 3; open triangles, cluster 4.

Results

The variation in elasticity could be summarized by four functional clusters (Fig. 2). The most obvious pattern of differentiation between these functional groups was a decrease in the relative importance of adult survival and an increase in the relative importance of fecundity from cluster 1 to cluster 4 (Table 1). The first cluster consisted of only lake sturgeon, a species with a unique life history and population dynamics that are expected to be most sensitive to changes in adult survival, as evidenced by its high adult survival elasticity and low juvenile survival and fecundity elasticities. The second cluster included nine species for which population dynamics are expected to be most sensitive to changes in juvenile and adult survivals and less sensitive to changes in fecundity. Examples include the paddlefish (*Polyodon spathula*), muskellunge (*Esox masquinongy*), and channel catfish (*Ictalurus punctatus*). The third cluster included 51 species for which population dynamics are expected to be most sensitive to changes in juvenile survival. Examples include grayling (*Thymallus arcticus*), longnose sucker (*Catostomus catostomus*), and yellow perch (*Perca flavescens*). The fourth cluster included 27 species for which population dynamics are expected to be most sensitive to changes in juvenile survivorship and fecundity. Examples include central mudminnow (*Umbra limi*), warmouth (*Lepomis gulosus*), and fantail darter (*Etheostoma flabelare*).

In terms of life-history traits, elasticity clusters 1 to 4 also tended to exhibit decreased age at maturity, longevity, and reproductive life span. Lake sturgeon exhibited the latest age at maturity and the longest life span and, consequently, the longest reproductive life span (60 years). Species in cluster 2

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Table 1. Elasticities of juvenile survival ($\varepsilon(S_J)$), adult survival ($\varepsilon(S_A)$), and fecundity ($\varepsilon(F)$) and mean values of age at maturity, longevity, reproductive life span, juvenile survivorship (l_J), adult survival (S_A), and mean fecundity (F) across the entire adult period for 88 species of North American freshwater fishes and four clusters determined from the variation in elasticities.

			Elasticities								
Species	Family	Cluster	$\varepsilon(S_J)$	$\varepsilon(S_A)$	$\varepsilon(F)$	Age at maturity	Longevity	Reproductive life span	l_J	S_A	Mean F
Acipenser fulvescens	Acipenseridae	1	0.23	0.72	0.05	20	80	60	0.0023	0.93	77 016
Polyodon spathula	Polyodontidae	2	0.44	0.45	0.1	9.5	30	20.5	0.0048	0.84	27 878
Lepisosteus osseus	Lepisosteidae	2	0.42	0.46	0.12	6	30	24	0.0033	0.79	27 830
Amia calva	Amiidae	2	0.47	0.4	0.13	5	24	19	0.0012	0.75	43 253
Salvelinus namaycush	Salmonidae	2	0.39	0.52	0.1	8	41	33	0.0062	0.85	3 951
Esox lucius	Esocidae	2	0.48	0.36	0.16	3	24	21	0.0012	0.64	32 000
Esox masquinongy	Esocidae	2	0.46	0.39	0.15	4	30	26	0.0011	0.72	125 260
Ictalurus punctatus	Ictaluridae	2	0.5	0.34	0.16	4	14	10	0.0031	0.64	7 977
Pylodictis olivaris	Ictaluridae	2	0.5	0.36	0.14	5	19	14	0.0027	0.725	9 100
Aplodinotus grunniens	Scianidae	2	0.51	0.33	0.16	4	17	13	0.0003	0.67	550 000
Mean			0.46	0.4	0.14	5.39	25.44	20.06	0.0027	0.74	91 917
Range			0.39–0.51	0.33–0.52	0.1–0.16	3–9.5	14–41	10–26	0.0003–0.0062	0.64–0.85	3 951 – 550 000
Hiodon tergisus	Hiodontidae	3	0.49	0.26	0.25	2	15	13	0.001	0.45	14 150
Dorosoma cepedianum	Clupeidae	3	0.52	0.22	0.26	2	14	12	0.0002	0.44	281 044
Coregonus artedi	Salmonidae	3	0.57	0.23	0.19	3	10	7	0.002	0.51	4 616
Coregonus clupeaformis	Salmonidae	3	0.48	0.27	0.24	2	28	26	0.002	0.51	67 892
Coregonus hoyi	Salmonidae	3	0.59	0.22	0.2	3	11	8	0.0032	0.53	7 580
Oncorhynchus nerka	Salmonidae	3	0.65	0.13	0.22	4	4	0	0.0014	0.35	450
Prosopium cylindraceum	Salmonidae	3	0.54	0.3	0.16	5	14	9	0.0045	0.68	6 460
Salvelinus fontinalis	Salmonidae	3	0.59	0.12	0.29	2	6	4	0.0097	0.235	1 210
Thymallus arcticus	Salmonidae	3	0.54	0.29	0.17	4	11	7	0.0018	0.6	5 500
Prosopium williamsoni	Salmonidae	3	0.53	0.29	0.18	3	17	14	0.0028	0.6	5 000
Salmo clarki	Salmonidae	3	0.63	0.17	0.2	4	7	3	0.0102	0.49	850
Esox americanus	Esocidae	3	0.58	0.13	0.29	2	7	5	0.005	0.29	4 318
Esox niger	Esocidae	3	0.61	0.18	0.2	3	9	6	0.0011	0.49	14 242
Pimephales notatus	Cyprinidae	3	0.63	0.06	0.31	2	3	1	0.0069	0.13	3 564
Rhinichthys atratulus	Cyprinidae	3	0.68	0.1	0.23	3	4	1	0.0035	0.29	2 674
Semotilus atromaculatus	Cyprinidae	3	0.63	0.16	0.21	3	8	5	0.0046	0.46	3 886
Gila atraria	Cyprinidae	3	0.59	0.22	0.19	4	10	6	0.0028	0.58	24 296
Richardsonius balteatus	Cyprinidae	3	0.6	0.1	0.3	2.5	5	2.5	0.0091	0.23	1 852
Carpoides carpio	Catostomidae	3	0.58	0.22	0.19	3.5	11	7.5	0.0005	0.56	154 149
Catostomus platyrhynchus	Catostomidae	3	0.6	0.2	0.19	4	9	5	0.0085	0.56	2 350
Ictiobus bubalus	Catostomidae	3	0.52	0.31	0.17	3.5	19	15.5	0.0003	0.65	233 031
Catostomus catostomus	Catostomidae	3	0.53	0.31	0.16	5	12	7	0.0037	0.66	13 600
Catostomus commersoni	Catostomidae	3	0.62	0.2	0.18	6	10	4	0.003	0.65	60 325
Ictiobus cyprinellus	Catostomidae	3	0.57	0.23	0.19	3.5	12	8.5	0.0004	0.59	400 000
Moxostoma anisurum	Catostomidae	3	0.62	0.19	0.18	5	9	4	0.0029	0.6	25 625
Moxostoma duquesnei	Catostomidae	3	0.59	0.22	0.2	3	10	7	0.0052	0.52	2 342

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Table 1 (*continued*).

Species	Family	Cluster	Elasticities			Age at maturity	Longevity	Reproductive life span	l_1	S_A	Mean F
			$\varepsilon(S_J)$	$\varepsilon(S_A)$	$\varepsilon(F)$						
Moxostoma erythrurum	Catostomidae	3	0.64	0.16	0.2	4	7	3	0.0022	0.49	16 689
Moxostoma macrolepidotum	Catostomidae	3	0.6	0.2	0.2	3	9	6	0.0021	0.49	20 325
Ameiurus melas	Ictaluridae	3	0.61	0.19	0.2	3	9	6	0.0044	0.49	3 069
Ameiurus natalis	Ictaluridae	3	0.65	0.14	0.22	3	7	4	0.0036	0.42	2 961
Ameiurus nebulosus	Ictaluridae	3	0.6	0.1	0.3	2.5	5	2.5	0.0049	0.25	5 075
Noturus flavus	Ictaluridae	3	0.57	0.15	0.28	2	9	7	0.0139	0.35	820
Lota lota	Gadidae	3	0.54	0.27	0.18	3	16	13	0.0002	0.59	628 808
Morone chrysops	Percichthyidae	3	0.55	0.18	0.27	2	9	7	0.0003	0.35	527 850
Ambloplites rupestris	Centrarchidae	3	0.57	0.24	0.19	3	13	10	0.0025	0.56	4 981
Lepomis gibbosus	Centrarchidae	3	0.57	0.15	0.28	2	8	6	0.0044	0.32	7 222
Lepomis macrochirus	Centrarchidae	3	0.59	0.21	0.2	3	9	6	0.0015	0.49	19 225
Lepomis megalotis	Centrarchidae	3	0.57	0.15	0.28	2	7	5	0.0057	0.29	3 361
Lepomis microlophus	Centrarchidae	3	0.58	0.13	0.29	2	8	6	0.0025	0.32	42 872
Micropterus dolomieu	Centrarchidae	3	0.56	0.26	0.19	3	14	11	0.0031	0.575	7 708
Micropterus salmoides	Centrarchidae	3	0.49	0.26	0.25	2	15	13	0.0031	0.45	25 544
Pomoxis annularis	Centrarchidae	3	0.56	0.16	0.28	2	8	6	0.0015	0.32	39 922
Pomoxis nigromaculatus	Centrarchidae	3	0.56	0.16	0.28	2	9	7	0.0014	0.35	75 590
Centrarchus macropterus	Centrarchidae	3	0.57	0.15	0.28	2	8	6	0.0027	0.32	11 699
Lepomis auritus	Centrarchidae	3	0.57	0.14	0.29	2	8	6	0.0065	0.32	3 879
Micropterus punctulatus	Centrarchidae	3	0.54	0.18	0.27	2.5	8	5.5	0.0028	0.4	11 911
Perca flavescens	Percidae	3	0.58	0.23	0.19	3	12	9	0.001	0.55	43 680
Percina caprodes	Percidae	3	0.63	0.06	0.31	2	4	2	0.0044	0.15	2 214
Percina maculata	Percidae	3	0.63	0.05	0.32	2	4	2	0.0109	0.15	1 378
Stizostedion canadense	Percidae	3	0.57	0.27	0.17	5	12	7	0.0015	0.66	52 500
Stizostedion vitreum	Percidae	3	0.53	0.32	0.15	5	14	9	0.001	0.69	82 700
Mean			0.58	0.19	0.23	3	9.94	6.92	0.0036	0.45	58 490
Range			0.48–0.68	0.05–0.32	0.15–0.32	2–6	3–28	0–26	0.0002–0.0139	0.13–0.68	450 – 527 850
Dorosoma petenense	Clupeidae	4	0.48	0.03	0.48	1	2	1	0.0006	0.06	19 100
Umbra limi	Umbridae	4	0.47	0.06	0.47	1.5	4	2.5	0.0485	0.111	628
Clinostomus elongatus	Cyprinidae	4	0.47	0.06	0.47	1	4	3	0.036	0.09	818
Cyprinella spiloptera	Cyprinidae	4	0.47	0.06	0.47	1	4	3	0.0428	0.09	672
Notemigonus crysoleucas	Cyprinidae	4	0.42	0.16	0.42	1.5	9	7.5	0.0034	0.32	101 000
Notropis atherinoides	Cyprinidae	4	0.48	0.04	0.48	1	3	2	0.0234	0.071	2 164
Notropis hudsonius	Cyprinidae	4	0.48	0.05	0.48	1.5	3	1.5	0.0339	0.096	1 614
Notropis rubellus	Cyprinidae	4	0.48	0.04	0.48	1	3	2	0.028	0.071	1 932
Notropis stramineus	Cyprinidae	4	0.48	0.04	0.48	1	3	2	0.037	0.071	1 050
Lythrurus umbratilis	Cyprinidae	4	0.48	0.04	0.48	1	2	1	0.0366	0.06	1 659
Pimephales promelas	Cyprinidae	4	0.46	0.07	0.46	1	2	1	0.0149	0.06	4 260
Hybognathus nuchalis	Cyprinidae	4	0.48	0.04	0.48	1	3	2	0.0232	0.071	3 000
Lepomis cyanellus	Centrarchidae	4	0.45	0.1	0.45	1	6	5	0.0061	0.17	48 000

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Table 1 (*concluded*).

Species	Family	Cluster	Elasticities			Age at maturity	Longevity	Reproductive life span	l_1	S_A	Mean F
			$\varepsilon(S_1)$	$\varepsilon(S_A)$	$\varepsilon(F)$						
Lepomis gulosus	Centrarchidae	4	0.42	0.16	0.42	1	8	7	0.0046	0.26	52 935
Lepomis humilis	Centrarchidae	4	0.47	0.07	0.47	1	4	3	0.029	0.086	2 077
Elassoma zonatum	Centrarchidae	4	0.48	0.05	0.48	1	3	2	0.0607	0.071	300
Etheostoma julise	Percidae	4	0.48	0.04	0.48	1	3	2	0.064	0.071	396
Etheostoma lepidum	Percidae	4	0.47	0.06	0.47	1	4	3	0.05	0.086	385
Percina sciera	Percidae	4	0.47	0.06	0.47	1	3	2	0.066	0.071	268
Etheostoma blennioides	Percidae	4	0.47	0.06	0.47	1	5	4	0.0407	0.1	747
Etheostoma caeruleum	Percidae	4	0.47	0.05	0.47	1	4	3	0.036	0.086	876
Etheostoma exile	Percidae	4	0.47	0.05	0.47	1	4	3	0.0447	0.086	1 134
Etheostoma flabellare	Percidae	4	0.47	0.06	0.47	1	5	4	0.0695	0.1	456
Etheostoma nigrum	Percidae	4	0.47	0.06	0.47	1	5	4	0.0915	0.1	230
Etheostoma olmstedi	Percidae	4	0.48	0.04	0.48	1	3	2	0.04	0.071	746
Etheostoma spectabile	Percidae	4	0.48	0.04	0.48	1	3	2	0.0289	0.071	2 076
Percina phoxocephala	Percidae	4	0.48	0.05	0.48	1	3	2	0.029	0.071	825
Mean			0.47	0.06	0.47	1	3.89	2.83	0.0366	0.1	9 235
Range			0.42–0.48	0.03–0.16	0.42–0.48	1–1.5	2–9	1–7.5	0.0006–0.0915	0.06–0.32	230 – 101 000

were characterized by medium to high values of age at maturity (3–9.5 years), moderate to long life spans (14–41 years), and reproductive life spans of 10–26 years. Species in cluster 3 were characterized by medium values of age at maturity (2–6 years), short to moderate life spans (3–28 years), and reproductive life spans of 0 (semelparous species) to 26 years. Cluster 4 consisted mainly of early-maturing species (1–1.5 years) with short life spans (2–9 years) and reproductive life spans of 1–7.5 years.

Results of analysis of variance indicated that clusters differed significantly in values of elasticity and life-history traits (Table 2). Clusters 2 and 3 differed significantly in all three vital rate elasticities and also in age at maturity, reproductive life span, and longevity. Strong significant differences between clusters 3 and 4 were found for all traits examined and all elasticity estimates. Clusters 2 and 4 also showed strong significant differences in all traits and elasticity parameters with the exception of juvenile survival elasticity. Significant differences between traits were not necessarily reflected in significant differences between corresponding elasticities. For example, estimates of juvenile survivorship were different between clusters 2 and 4, even though juvenile survival elasticities were not significantly different. Similarly, elasticities of fecundity and juvenile survival were significantly different between clusters 2 and 3, whereas the corresponding trait means for fecundity and juvenile survivorship were not.

Relationships between life-history traits and elasticities

According to the life-history strategies identified by Winemiller and Rose (1992, 2005), lake sturgeon is an extreme case of periodic strategist with low relative fecundity. Cluster 2 includes periodic strategists and another extreme strategist, *Aplodinotus grunniens*, with early age at maturity and very high mean fecundity. Cluster 3 encompasses some intermediate strategists with early maturity and high mean fecundity, as well as some species leaning toward the periodic strategy. Cluster 4 is represented by opportunistic and equilibrium strategists. A triangular plot of juvenile survivorship, age at maturity, and fecundity rates (Fig. 3) better illustrates that primary life-history strategies did not always correspond to categories based on elasticity patterns, as indicated by the aggregation of representative species of clusters 2, 3, and 4 in the center of the triangular plot.

The absence of a stronger alignment between elasticity clusters and primary life histories can be partly explained by the correlation patterns exhibited between elasticities and life-history traits. First, in several instances, significant correlations between two traits were also significant between the first trait and corresponding elasticity of the second trait, but the relationships were in opposite directions (Table 3). For example, age at maturity was negatively associated with juvenile survivorship (r_S = –0.62) but positively associated with the elasticity of juvenile survival (r_S = 0.40). This means that the probability of surviving the juvenile period and becoming an adult increases in early-maturing fishes, which increases the relative importance of juvenile survival on fitness as age at maturity increases. Nevertheless, species maturing later than age 3 (less than 20% of the species in this study) seem to exhibit a negative association between age at maturity and the relative importance of juvenile sur-

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Table 2. *P* values from Mann–Whitney *U* tests used to compare age at maturity, reproductive life span, mean fecundity (F), juvenile survivorship (l_1), longevity, and elasticity for juvenile survival ($\varepsilon(S_J)$), adult survival ($\varepsilon(S_A)$), and fecundity ($\varepsilon(F)$) between multispecies clusters.

	Cluster 2 vs. cluster 3	Cluster 3 vs. cluster 4	Cluster 2 vs. cluster 4
Age at maturity	<0.0001*	<0.0001*	<0.0001*
Reproductive life span	<0.0001*	<0.0001*	<0.0001*
F	0.08	<0.0001*	<0.0001*
l_1	0.5	<0.0001*	<0.0001*
Longevity	<0.0001*	<0.0001*	<0.0001*
Elasticity			
$\varepsilon(S_J)$	<0.0001*	<0.0001*	0.89
$\varepsilon(S_A)$	<0.0001*	<0.0001*	<0.0001*
$\varepsilon(F)$	<0.0001*	<0.0001*	<0.0001*

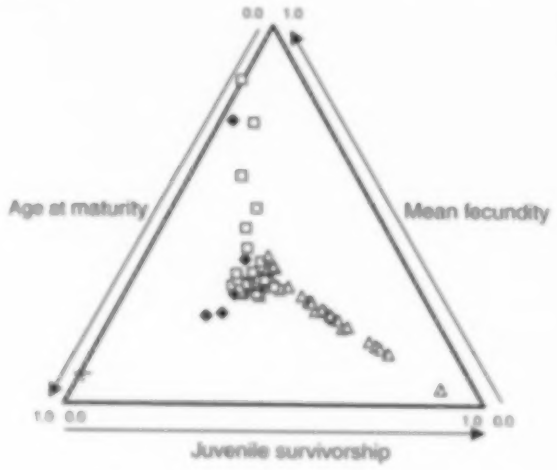
Note: Asterisks indicate comparisons where the observed *P* is less than the Bonferroni-corrected value of 0.017.

vival (Fig. 4*a*), which can be interpreted as a trade-off between adult survival and the relative importance of juvenile survival (i.e., its elasticity) as fish mature later in life. Age at maturity also showed a strong negative relationship with fecundity elasticity (r_S = –0.95) but a positive relationship with fecundity itself (r_S = 0.54), indicating that fishes maturing late in life generally produce more offspring per year, but their population dynamics are relatively insensitive to changes in fecundity rates. These results are reflected in the negative association between fecundity as a trait and its elasticity (r_S = –0.63).

Second, in other instances, a significant correlation between two traits was not paralleled by the relationship between the first trait and corresponding elasticity of the second trait. For example, despite the fact that fecundity is strongly associated with juvenile survivorship (r_S = –0.86), fecundity was not significantly related to the elasticity of juvenile survival (r_S = 0.08). Furthermore, a trait can be strongly associated with elasticities other than its own corresponding elasticity. For example, the correlation between juvenile survivorship and the elasticity of juvenile survival was low and not significant (r_S = –0.34), in spite of juvenile survivorship exhibiting a strong negative association with the elasticity of adult survival (r_S = –0.70) and a strong positive association with the elasticity of fecundity (r_S = 0.68).

Reproductive life span and longevity are highly correlated and both played an important role in the determination of the elasticities of adult survival and fecundity, although in opposite directions. Long-lived fishes with long reproductive life spans are generally more fecund, as indicated by the positive association between reproductive life span and fecundity (r_S = 0.70), which causes a decline in the relative importance of fecundity and increases the elasticity of adult survival in old fish (Fig. 4*b*). In spite of the significant negative correlation between reproductive life span and juvenile survivorship, changes in reproductive life span do not seem to be associated with changes in the elasticity of juvenile survival. The same is true for longevity, which also exhibited the greatest trait association with the elasticity of adult survival (r_S = 0.97). Despite this strong relationship, the relative importance of adult survival was predominant over all other factors in only the lake sturgeon, whereas population growth rates of species in cluster 2 are equally sensitive to perturbations in juvenile or adult survival. In fact, most of the long-lived fishes in cluster 2 (e.g., *Amia calva*, *Esox lucius*, and *Esox masquinongy*) were most sensitive to changes in juvenile survival.

Fig. 3. The distribution of elasticity patterns, as indicated by clusters, within the life-history space determined by 0–1 scaled traits juvenile survivorship, age at maturity, and mean fecundity across the entire adult period (annual number of female offspring per female). Each point represents a species. The type of point indicates the species' cluster affiliation: plus sign, cluster 1; solid diamonds, cluster 2; open squares, cluster 3; open triangles, cluster 4.



Life-history predictors of elasticities

Age at maturity, reproductive life span, fecundity, juvenile survivorship, and longevity were all significantly correlated with the elasticities of adult survival and fecundity, but in opposite directions. However, best life-history predictors of elasticity patterns were longevity, which explained 93% of the variability in the elasticity of adult survival ($P < 0.01$):

$$\varepsilon(S_A)_{arcs} = 12.387 \ln(t_{max}) - 1.57$$

and age at maturity, which explained 92% of the variability in the elasticity of fecundity ($P < 0.01$):

$$\varepsilon(F)_{arcs} = -12.419 \ln(\alpha)$$

where $\varepsilon(S_A)_{arcs}$ and $\varepsilon(F)_{arcs}$ are the arcsine square root transformed elasticity values of adult survival and fecundity, respectively.

The absence of significant life-history predictors of juvenile survival elasticity can be solved by taking advantage of the additive-to-unit property of elasticities, estimated as $\varepsilon(S_J) = 1 - [\varepsilon(S_A) + \varepsilon(F)]$. In the absence of detailed knowledge of a species' life history, information on life span and age at first maturity can be used to approximate the elasticities of fecundity, juvenile survival, and adult survival and in-

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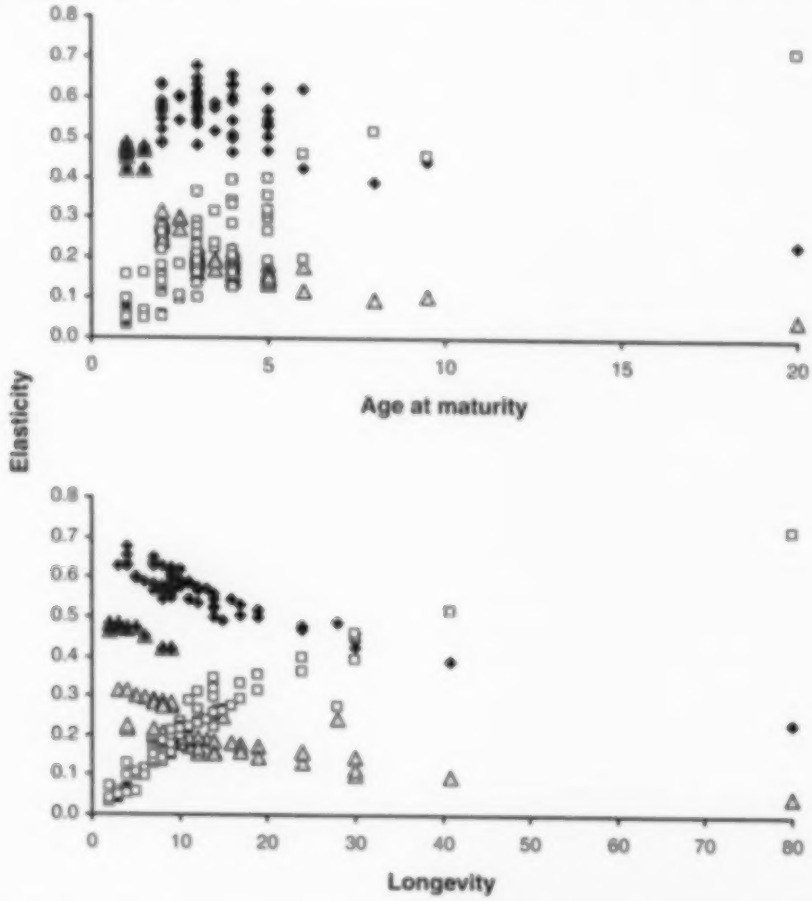
Table 3. Spearman rank correlation coefficients summarizing the relationships between the elasticities for juvenile ($\varepsilon(S_J)$) and adult ($\varepsilon(S_A)$) survival and fecundity ($\varepsilon(F)$) and the life-history traits used to estimate the elasticities.

	Elasticities						
Trait	$\varepsilon(S_J)$	$\varepsilon(S_A)$	$\varepsilon(F)$	Longevity	l_J	F	Reproductive life span
Age at maturity	0.40*	0.85*	–0.95*	0.77*	–0.62*	0.54*	0.61*
Reproductive life span	–0.12	0.91*	–0.79*	0.97*	–0.63*	0.7*	—
F	0.08	0.7*	–0.63*	0.71*	–0.86*	—	—
l_J	–0.34	–0.7*	0.68*	–0.69*	—	—	—
Longevity	0.03	0.97 *	–0.9 *	—	—	—	—

Note: Asterisks indicate correlation coefficients for which the observed P is less than the critical P of 0.002 obtained after Bonferroni adjustment for multiple tests.

Fig. 4. Relationships between elasticities estimated for fecundity, juvenile survival, and adult survival and (*a*) age at maturity and (*b*) longevity. Each point represents a species. Symbols denote each form of elasticity: shaded triangles, fecundity; solid diamonds, juvenile survival; open squares, adult survival.



fer the population dynamics and demographic sensitivity of the population in question.

Taxonomic affiliations

The nested analysis of variance identified genera within family or family within order as the taxonomic levels exhibiting the greatest variation in elasticity values and therefore most appropriate for the analysis of taxonomic affiliations of elasticity clusters. Analyzing orders, rather than families, resulted in many fewer points for the analysis, thus we analyzed genera within family (Table 4). Average elasticities for a genus were not good predictors of the elasticities of

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other genera within the same family, and therefore data points can be reasonably treated as independent. Nevertheless, species within genera do not add substantial variance that is independent of taxonomic association and are therefore not adequate for testing taxonomic affiliations of elasticity clusters.

From the 49 genera grouped in the three multispecies clusters, eight had memberships in cluster 2, 30 in cluster 3, and 13 in cluster 4. Eleven of the 17 families included in the analysis were represented by only one genus, and they exhibited memberships in all four elasticity clusters (Table 5). For the six taxonomic families for which we had life-history data for more than one member of the genus (Ictaluridae, Salmonidae, Catostomidae, Cyprinidae, Percidae, and Centrarchidae), all but Catostomidae had genera assigned to two elasticity clusters. The four ictalurid genera and the seven salmonid genera were distributed between clusters 2 and 3, and the 12 cyprinid genera, the five percid genera, and the seven centrarchid genera were distributed between clusters 3 and 4. Catostomidae was the only family in which all genera were assigned to a single cluster (cluster 3). Still, for taxonomically rich families such as Cyprinidae, Salmonidae, and Centrarchidae, the majority of genera tended to be assigned to a single cluster.

Results of the contingency table analysis indicated that elasticity clusters were dependent on taxonomic affiliations, suggesting that life histories of North American freshwater fishes promote preferred elasticity patterns for genera within families. The clearest examples of this relationship are given by the families Catostomidae and Salmonidae, with 100% of the genera in the former and 86% of the genera in the latter exhibiting the elasticity pattern portrayed by cluster 3.

Discussion

This study makes five important contributions. First, it demonstrates that interspecific variation in sensitivity of population growth rates to changes in the vital rates in North American freshwater fishes can be represented effectively by four functional groups. Second, the functional groups are distinguished by a trade-off between the elasticities of fecundity and adult survival, ranging from species exhibiting high sensitivity to perturbations in adult survival and low sensitivity to perturbations in fecundity rates (e.g., lake sturgeon) to species exhibiting low sensitivity to perturbations in adult survival and high sensitivity to perturbations in fecundity (e.g., *Etheostoma* sp., *Notropis* sp.). Third, elasticity patterns cannot be inferred in a straightforward manner from trade-offs between life-history traits, but the sensitivity of a population's growth rate to changes in adult survival and fecundity can be predicted empirically from life span and age at maturity, respectively. Fourth, elasticities are highly conserved among genera within the same taxonomic family. Fifth, there are key divergences between the elasticity patterns of freshwater fishes and those of other vertebrate taxa, namely opposite relationships between the elasticity of adult survival and the traits age at maturity, fecundity, and juvenile survivorship.

The segregation of species into ecologically different groups in clusters 1–4 provides a useful starting point for categorizing demographic patterns of freshwater fishes and analyzing broad interspecific differences in the contributions that vital rates make to population growth rates. Our study demonstrated that North American freshwater fishes cluster into well-differentiated groups based on particular combinations of fecundity, survival rates, age at maturity, and longevity. Nonetheless, we recognize that any attempt to categorize species into discrete groups, aiming to facilitate the management of species and populations that actually lie along a continuum, is somewhat artificial and can potentially oversimplify the management of an individual species. In general, population dynamics captured in the elasticity patterns of the species in this study indicate that the negative correlation between the elasticities of fecundity and adult survival across a wide range of life histories represents a genuine trade-off that is not paralleled by a similar correlation between related traits and that plays a central role in the demographic patterns and evolutionary biology (see Shea et al. 1994; Grant 1997; Grant and Benton 2000) of North American freshwater fishes.

Our results concur with the findings of Silvertown et al. (1993) and Shea et al. (1994) that the trade-offs between traits do not necessarily lead to trade-offs between elasticities related to those traits and, therefore, do not imply an exchange of contributions to fitness. However, life-history trade-offs might still reflect compensatory mechanisms (e.g., Van Winkle et al. 1993) that cannot be discerned from elasticities. In the present study, a strong trade-off between adult survival and juvenile survivorship is not reflected in a similar relationship between the elasticities of juvenile and adult survival. Furthermore, in our study, although the primary elasticity trade-off demonstrated the strong negative association between the elasticities of fecundity and adult survival rates ($r_S = -0.96$), the relationship between traits is opposite to that between elasticities and therefore does not represent a trade-off. Clearly, life-history strategies defined by trade-offs among traits are generally not analogous to elasticity patterns depicted by clusters. The lack of a consistent pattern in the mapping of primary life-history strategies and elasticity clusters was also found by Rose (2005), admitting that the magnitude of population responses to changes in fecundity and survival does not necessarily map onto the life-history surface. Nonetheless, the classification of life-history strategies based on the ability of populations and species to deal with environmental variation still allows complementary and alternative insights to management recommendations derived from deterministic models.

The importance of age at maturity and longevity in defining elasticity patterns has been shown in a number of studies of non-fish taxa (e.g., Cunnington and Brooks 1996; Heppell et al. 2000; Saether and Bakke 2000). For example, Heppell et al. (2000) generally found that elasticity patterns are strongly determined by age at maturity and specifically that the relative magnitude of fecundity elasticities in mammals can be predicted from this trait. Additionally, both age at maturity and longevity were strongly and positively correlated, as reported elsewhere (Read and Harvey 1989; Promislow and Harvey 1990; Hutchings 1993). We also found a strong correlation between longevity and the elasticity of fecundity. The negative associations between fecundity elasticity and both life span and age at maturity suggest that more resilient species begin reproduction at a later age and may also reflect the relaxation of selective pressure (see

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Table 4. Percentages of the variances in elasticities for juvenile survival ($\varepsilon(S_J)$), adult survival ($\varepsilon(S_A)$), and fecundity ($\varepsilon(F)$) that are attributable to different levels of taxonomy.

	Variances attributable (%)		
Elasticity	Species within genera	Genera within families	Family within orders
$\varepsilon(S_J)$	11.38	41.5	47.12
$\varepsilon(S_A)$	13.91	38.55	47.54
$\varepsilon(F)$	13.05	38.03	48.92

Note: Elasticities were arcsine square root transformed before analysis.

Grant and Benton 2000) on fecundity as age at maturity and life span increase.

Our results also suggest that general conclusions of previous studies (e.g., Crouse et al. 1987; Brault and Caswell 1993; Heppell and Crowder 1998), indicating that the population growth rates of long-lived species are most sensitive to changes in adult survival, generally apply to North American freshwater fish. This is clearly the case for *A. fulvescens*, and among the long-lived species of our study grouped in cluster 2, paddlefish (*Polyodon spathula*), longnose gar (*Lepisosteus osseus*), and lake char (*Salvelinus namaycush*) also exhibited dominant adult survival elasticities. The remaining species of this cluster were most sensitive to changes in juvenile survival, but the relative importance of adult survival was still high. Gross et al. (2002) found a similar pattern in their study of the sturgeon species *A. brevirostrum* (t_{max} = 37), *A. oxyrinchus* (t_{max} = 60), and *A. transmontanus* (t_{max} = 100), where according to their age-structured model, the elasticity of juvenile survival in *A. transmontanus* was greater than its adult survival elasticity. For the other two sturgeon species, adult survival elasticity was just slightly higher than that for juvenile survival (Gross et al. 2002). In general, our study shows that the relative importance of adult survival governs the population growth rates of fishes with life spans of 30 years or more, while other long-lived fishes with life spans shorter than 30 years have population growth rates slightly more sensitive to changes in juvenile survival rates.

The relatively strong taxonomic affiliation of elasticities observed in freshwater fishes contrasts with findings from surveys of mammals by Heppell et al. (2000) (to our knowledge, there are no studies reporting on relationships between elasticities and taxonomic affiliations for other vertebrate taxa) in which elasticities were found to be independent of phylogeny but correlated within taxonomic groups to the extent that life-history traits such as age at first reproduction often correlate within taxonomic groups. Similarly, Oli and Dobson (2003) found that a substantial proportion of the variation in elasticities was associated with phylogeny in 142 species of mammals, whereas the influence of phylogeny on elasticity patterns was minor. Despite our results suggesting that taxonomic affiliation could be used to estimate the elasticity patterns of poorly studied species when life-history data are unavailable, we still concur with Heppell et al. (2000) that life-history correlates should be more important than taxonomic relationships in predicting elasticities and guiding management efforts given the high proportion of variability in elasticities explained by longevity and age at maturity, but for species with poor life-history data, the elasticities of a relative (e.g., other genus within the same family) can be used as a first approach.

Comparisons of our results and those of other studies indicate that there are at least four patterns of variation in life history and demographic traits of freshwater fishes that are general to mammals, birds, turtles, and sharks. In all of these taxa, adult survival rates increase with age at maturity, adult survival elasticity increases with longevity, the elasticity of fecundity rate decreases with increased age at maturity, and in general, high adult survival elasticities and low fecundity elasticities are exhibited by long-lived species (Cunnington and Brooks 1996; Saether and Bakke 2000; Cortés 2002).

However, North American freshwater teleost fishes diverge from life-history – demographic patterns of mammals, birds, and sharks in several ways (there are not enough data to characterize these relationships in turtles). First, in fishes, the relative importance of adult survival to fitness increases with fecundity but decreases with increments in juvenile survivorship. Conversely, the elasticity of adult survival decreases with increased fecundity in mammals and birds and increases with juvenile survivorship in mammals. Second, in freshwater teleosts, the sensitivity of the population growth rate to perturbations in adult survival increases with age at maturity. However, in birds and sharks, population growth rates of early-maturing species are more sensitive to perturbation affecting adult survival than are late-maturing species. Hence, a strong, positive relationship between age at maturity and adult survival rates observed in teleosts, sharks, birds, and mammals translates into a positive correlation between age at maturity and the elasticity of adult survival in teleosts, but not in the other three taxa. Third, despite the dome-shaped relationship between age at maturity and juvenile survival elasticities characterizing the fishes in our study, age at maturity is strongly and positively correlated with juvenile survival elasticities of mammals (Heppell et al. 2000) and sharks (Cortés 2002).

These differences between elasticity patterns of teleost fishes and other vertebrate taxa can be mainly attributed to the following factors: (*i*) compared with teleost fish, other taxa such as mammals, birds, sharks, and turtles exhibit much lower clutch size; and (*ii*) there is a positive association between fecundity and age in teleosts throughout the adult stage as a result of indeterminate growth, whereas fecundity rates in birds, mammals, sharks, and turtles can decrease with age (see Crouse et al. 1987; Brault and Caswell 1993; Heppell and Crowder 1998) or be independent of age (see Hitchcock and Gratto-Trevor 1997; Mollet and Cailliet 2002). These two factors become very important in shaping a positive association between the probability of annual adult survival and both fecundity and clutch size in fish (see Fig. 5), which is contrary to a theoretical negative correlation between clutch size or fecundity and adult survival believed to be one of the most general trade-offs among animal species (Sibly and Calow 1983).

The influence of fecundity and its increase with age in the differences between elasticity patterns of fish and other vertebrate taxa can be illustrated by conducting demographic simulations on the stage-structured matrices of one representative species from each elasticity cluster, a species with vital rate elasticities similar to the average for its cluster selected to represent each cluster. To demonstrate this point, we used

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Table 5. Family affiliations of species and genera assigned to the elasticity clusters.

			Elasticity cluster							
			Species				Genera			
Family	Species no.	Genera no.	1	2	3	4	1	2	3	4
Acipenseridae	1	1	1				1			
Polyodontidae	1	1		1				1		
Lepisosteidae	1	1		1				1		
Amiidae	1	1		1				1		
Scianidae	1	1		1				1		
Hiodontidae	1	1			1				1	
Gadidae	1	1			1				1	
Percichthydae	1	1			1				1	
Umbridae	1	1				1				1
Clupeidae	2	1			1	1			1	1
Esocidae	4	1		2	2			1	1	
Ictaluridae	6	4		2	4			2	2	0
Salmonidae	10	7		1	9			1	6	0
Catostomidae	10	4			10			0	4	0
Cyprinidae	15	12			5	10		0	5	7
Percidae	16	5			5	11		0	3	2
Centrarchidae	16	7			12	4		0	5	2
Total	88	50	1	9	51	27	1	8	30	13

Note: Numbers in the table indicate the number of species and genera per family and per cluster family.

the minimum fecundity (i.e., fecundity at the age of first maturity) of each of the four representative species (lake sturgeon (cluster 1), muskellunge (cluster 2), yellow perch (cluster 3), and central mudminnow (cluster 4)) and fixed it throughout all the adult ages, and then we reduced this fecundity by different percentages (25%, 50%, 75%, 95%, and 99%) and adjusted the value of yearling survival in each simulation to preserve equilibrium ($\lambda = 1$). Reducing fecundity and increasing yearling survival to keep equilibrium produced a species-specific increase in adult survival elasticity with juvenile survivorship at about 85% clutch size reduction in lake sturgeon, 75% in muskellunge and yellow perch, and 99% in central mudminnow. Similarly, clutch size reductions produced a species-specific decrease in adult survival elasticity with fecundity at about 80% clutch size reduction in sturgeon, 65% in muskellunge, 75% in yellow perch, and 95% in central mudminnow. Across species, reductions in clutch size produced considerable reductions in the negative regression coefficient between juvenile survivorship and adult survival elasticity, from a steep slope in the line representing original matrices (i.e., fixed minimum fecundity but no reduction) to a gentle slope in the line representing those matrices incorporating 99% fecundity reductions (Fig. 6). This simulation shows that removing increases in fecundity associated with age and then reducing fecundity by several orders of magnitude can produce associative patterns between adult survival elasticity and both fecundity and juvenile survivorship that roughly resemble those of other vertebrate taxa. It was also evident from this simulation that substantial increments in yearling survival are necessary to preserve equilibrium in the population growth rate. These increments in yearling survival were similar for representative species of clusters 1, 2, and 3, but a large compensation in yearling survival was necessary to achieve equilibrium in the species representing the elasticity pattern portrayed by cluster 4, which comprises those species with population growth rates that are strongly sensitive to fecundity changes.

The life-history continuum, from highly reproductive species at one end to survivor species at the other end, as noted for plants (Franco and Silvertown 1996), birds (Saether 1988), and mammals (Purvis and Harvey 1995; Heppell et al. 2000) is not evident in freshwater fishes because in general those fish species exhibiting greater adult survival also exhibit greater fecundity. It seems that in fishes the main counterpart for increments in adult survival is not a decrease in fecundity or clutch size but a strong trade-off with juvenile survivorship. Moreover, potential differences in population dynamics of fish and other vertebrate taxa attributed to differences in clutch size of several orders of magnitude are further diminished by a strong negative correlation between fecundity and juvenile survivorship in fish. Nevertheless, there is an elasticity continuum represented by species with a high contribution of fecundity rates to the population growth rate at one end and species with a high contribution of adult survival rates to the population growth rate at the other end. And this elasticity continuum is common to all the above-mentioned vertebrate taxa, as well as plants. Hence, the possibility that this could be a general pattern among many different taxa suggests that some elasticity patterns can be preserved in spite of large differences in life histories, and therefore, it could be of greater relevance than life-history patterns to predict population dynamics and to evaluate the effect of anthropogenic perturbations on population's fitness. In addition, this particular trade-off between the elas-

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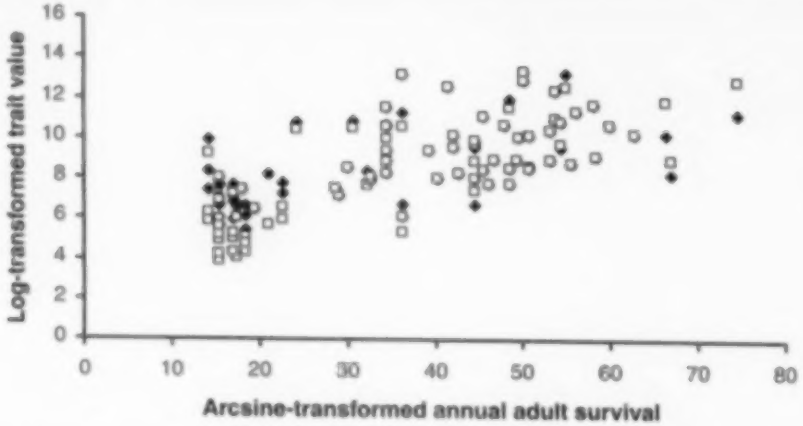
Fig. 5. Relationships between adult survival and both mean fecundity (solid diamonds) and mean clutch size (open squares). Each point represents a species.



ticities of adult survival and fecundity across a wide range of life histories also suggests that this continuum could be one of the main axes in the evolution of life histories (see Shea et al. 1994), because the demographic machinery that produces changes in population size is essential in all processes that underlie the dynamics and regulation of populations (Dobson and Oli 2001), and the sensitivity of λ to changes in individual vital rates may also reflect the strength of selection on different points of the life cycle (Grant 1997; Van Tienderen 2000).

Scope and limitations of the analysis

Elasticity patterns are not a forecast tool because the dynamics of any wild population are influenced by many factors not considered in our models, and population projections derived from elasticities assume that vital rates are constant and that population dynamics lead to stable-stage distributions. In addition, elasticity values have been determined from average values of life-history traits, and populations may differ in these traits as a consequence of local adaptations. Nevertheless, management recommendations derived from elasticity patterns can be optimized with the incorporation of more precise and reliable estimates of life-history traits and their variation obtained from long-term studies of the actual populations of interest.

Ranking the relative importance of vital rates through elasticity analysis does not account for important ecological processes such as density dependence or demographic and environmental stochasticity (see Lande et al. 1994). Nevertheless, the sensitivity of λ to changes in a given trait is generally inversely related to the temporal variation of this trait (Ehrlén and Van Groenendael 1998; Pfister 1998; De Kroon et al. 2000), meaning that those demographic parameters with the greatest influence in the population growth rate are expected to be the least variable because a reduction in the variance of sensitive traits seems to be favored through selection (Saether and Bakke 2000). Density-dependent compensation and temporal variation can be incorporated into demographic modeling; however, the quality of our data limited our ability to employ stochastic approaches. In addition, demographic parameters from deterministic perturbation analysis have been found to be highly correlated with demographic indices estimated with stochastic models (Neubert and Caswell 2000), and elasticities of λ calculated with density-independent, time-invariant models effectively predict changes in population growth derived from models that incorporate density dependence and environmental or demographic stochasticity when populations are below from the equilibrium size (Grant and Benton 2000). In any case, results of the present analysis can have important connotations when the assumptions and restrictions of the deterministic model used herein favor qualitative ranking of vital rate elasticities, guiding management options, avoiding the temptation of overinterpreting quantitative differences (see De Kroon et al. 2000).

Changes in development time (including changes in age at maturity) in populations with high fecundity rates and low survival can have greater effects (one order of magnitude) on population growth than changes in fecundity (see Lewontin 1965; MacArthur and Wilson 1967). Thus, arguing that levels of uncertainty in age at maturity and longevity for North American freshwater fishes are much lower compared with other traits (e.g., Scott and Crossman 1973) and considering that our results identify these traits as explaining most of the variation in the elasticities of adult survival and fecundity, extra caution should be taken when using elasticities in species exhibiting important variation in age at maturity or longevity among populations.

Unreliable life-table parameters can produce misleading evaluations of demographic vulnerability, and therefore it is important to find methods to detect them before any attempt to calculate elasticities. The use of S_0 (survivorship from birth to age 1) as a demographic filter of faulty parameters in our database was useful in this aspect. The estimate of S_0 in any demographic and population viability analysis is undoubtedly a major source of uncertainty in life-table computations. The approximation of S_0 at equilibrium bypasses the difficulty in empirically estimating this parameter and reduces this methodological limitation, although it is also necessary to assume that the other elements of the life table are moderately reliable.

The artifice of tuning the model to $\lambda = 1$ may be seen as a drawback when there is ample evidence that many fish populations exhibit high population growth rates at low abun-

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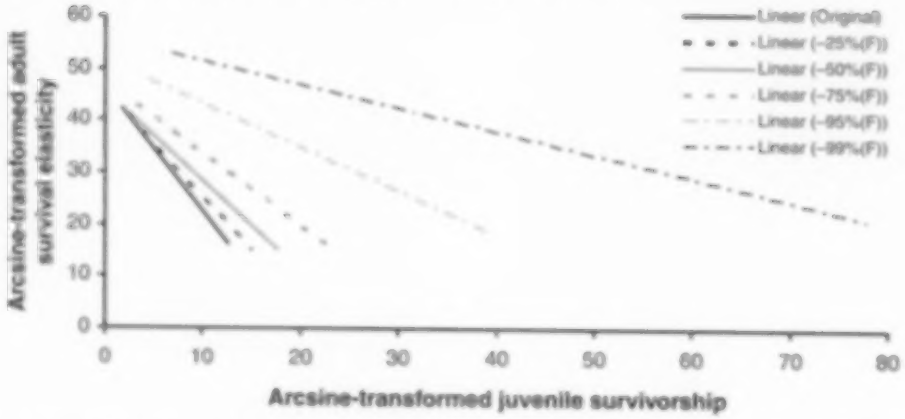
Fig. 6. The effects of reductions in clutch size (25%, 50%, 75%, 95%, and 99%) over the relationship between adult survival elasticity and juvenile survivorship across representative species lake sturgeon (*Acipenser fulvescens*), muskellunge (*Esox masquinongy*), yellow perch (*Perca flavescens*), and central mudminnow (*Umbra limi*). The "original" line represents results for base-run matrices incorporating the minimum clutch size, fixed throughout all the adult ages, for each species. *F*, fecundity.



dance (e.g., Myers 2001). However, this step not only served as a mean of standardizing individual matrices by canceling the effect of population state on predicted responses to perturbations, but also the qualitative ranking of elasticities is expected to be preserved because actual population growth rates have little influence on the pattern of elasticities (Oli and Dobson 2003). Rose (2005), working with 10 fish populations, found that population state can affect the magnitude, but not the general patterns, of population responses among matrix-model species.

Additionally, the fact that adult survival was estimated as a function of mean age at maturity and longevity and that juvenile survival was calculated from estimates of adult survival carries the risk of one faulty parameter affecting several other trait values, consequently reducing the effectiveness of any management strategy based on the calculated elasticities. At any rate, the use of deterministic matrix models in population management has grown considerably since the development of the concept of elasticity (Beissinger and Westphal 1998), in part because changes in elasticities derived from faulty parameters are easily computable from projection matrices (Caswell 1996) and as more precise data on survival and fecundity rates become available they can be readily incorporated into the model.

Management applications

The present study provides a useful guideline to questions about the sensitivity of population growth rates of North American fishes to changes in the vital rates produced by management or conservation actions. Potential applications of the present elasticities summarization can include conservation of endangered species, decision analysis concerned with the effects on fish communities caused by specific management actions, stocking programs, habitat-restoration projects, fisheries management, and the development of demographic indices.

For instance, lake sturgeon (*Acipenser fulvescens*), which is one of the principal species at risk in the Great Lakes (Anonymous 1974), exhibits an elasticity pattern indicating that adult mortality, e.g., as caused by fishing, can have a much larger negative impact on the population growth rate of this species relative to a proportional impact on fecundity through, for instance, instream barriers that impede the sturgeon's spawning migration. Conversely, dams and barriers can have a large negative impact on the population dynamics of percids and cyprinids exhibiting potamodromous behavior because their sensitivity to perturbations in fecundity rates makes them highly vulnerable to any action reducing their reproductive success.

Elasticity clusters can improve management recommendations derived from life-history strategies. For example, Winemiller and Rose (1992) recommended maintenance of critical densities of adult stocks and protection of spawners and spawning habitats as crucial strategies in the management of long-lived "periodic" fishes, which is in agreement with our results that maintenance of adult survival rates is important for the management of these species. Protection of spawners and spawning habitats should be important for any fish population, yet perturbations to fecundity rates only have a moderate effect on the population dynamics of periodic strategists. Conversely, the analysis of primary life-history strategies indicates that this management strategy (protection of spawning habitat) is crucial for opportunistic strategists, in agreement with our results. Nonetheless, the preservation of juvenile survival rates is as important as the maintenance of fecundity rates in these fishes, as indicated by the relative importance of juvenile survival in the population dynamics of species exhibiting the elasticity pattern of cluster 4. Equilibrium strategists should be sensitive to changes in juvenile survivorship and fecundity because there is a premium on successful low-level recruitment during each spawning period for these species (Winemiller 2005). This is in concordance with our findings for species in cluster 4, but this cluster also includes all opportunistic strategists. Several of the fish species in this study exhibited intermediate life-history strategies underpinning population growth rates highly sensitive to perturbations in juvenile survival rates, and consequently they all had memberships in cluster 3. These examples demonstrate that the analysis of primary life-history strategies and the study of elasticity patterns are complemen-

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tary approaches for guiding management actions. For instance, although equilibrium and opportunistic strategists exhibit overlapping elasticity patterns, these two strategies react differently to natural and human-induced environmental perturbations on the basis of primary life-history strategies.

Adult stocking programs can increase adult survival rates, fecundity rates, or both. Stocking of adult salmonids can have moderate effects on the population growth rate (by reducing adult fishing mortality rates) if they are not reproducing successfully in the wild, but the contribution of stocked adults can be substantial and promote self-sustaining populations if reproduction is successful, because the relative importance of fecundity in the vast majority of salmonids can be as important as that of adult survival. Similarly, stocking juveniles, as a maneuver to compensate for high juvenile mortality, can have a greater positive effect on the population growth rates of salmonids if juveniles adapt well to the wild and reach the age of first maturity.

The fact that juvenile survival elasticity is relatively high in the large majority of North American freshwater teleosts indicates that the alteration of nursery habitats and those used by juveniles and the increase of competition by nonnative species having a negative impact on juvenile survival are major sources of demographic instability. Hence, conservation and restoration of juvenile habitat is of great importance in all North American freshwater teleosts; conservation and restoration of spawning habitats would be especially important for early-maturing species such as those included in cluster 4.

Other extensions of the use of elasticities for management purposes can include the application of demographic analyses to fisheries management seeking to estimate optimal harvesting (see Lande et al. 1994) or reference points (see Hayes 2000), where elasticity patterns can be useful by providing additional information on population responses to different fishing levels. Similarly, fecundity elasticities have been used as an index of the demographic sensitivity of potamodromous species to instream barriers impeding access to preferred spawning areas (L.A. Vélez-Espino and R.L. McLaughlin, unpublished data).

Finally, the summarization of species' population dynamics in elasticity patterns and the facilitation of elasticity estimates for species with minimal demographic data from functional groups, life-history traits such as age at maturity and longevity, or taxonomic affiliations provide helpful tools to conservation ecologists and resource managers attempting to anticipate the response of individual species to human alterations of the environment. We close expecting this contribution to be fruitful not only in the management arena where agencies are working to protect the fish diversity of North America, but also in the research contact zone between life history, demography, and evolutionary biology.

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Longitudinal gradients in threshold sizes for alternative male life history tactics in a population of Atlantic salmon (*Salmo salar*)

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Abstract: Atlantic salmon (*Salmo salar*) males may mature early in life in freshwater, rather than maturing after a migration to sea, if their size is above a threshold value. We analyzed the spatiotemporal variation in size and incidence of the early maturity tactic among males over an 8-year period in six subpopulations on two branches of a river and collected environmental data on each site and across the river scape. A positive longitudinal trend in the frequency of early maturing males that was stable over the 8-year period occurred from the mouth to the head of the river. Threshold sizes for early maturation varied among subpopulations; size thresholds for male parr to mature were higher in downstream habitats and lowest upstream. This pattern was consistent in both river branches over the 8-year period and was not related to either the density of parr or site-specific abiotic habitat characteristics. However, the cumulative incidence of habitat features that could impede migration of large individuals increased with increasing upstream distance. Migration costs may contribute to the observed variation in threshold sizes.

Résumé : Les mâles du saumon atlantique (*Salmo salar*) peuvent atteindre la maturité sexuelle tôt dans la vie en eau douce au stade de tacon, plutôt qu'après une migration en mer, si leur taille est supérieure à une valeur seuil. Nous avons analysé la variation spatio-temporelle de la taille et de l'incidence de la stratégie de maturité hâtive chez les tacons mâles sur une période de 8 ans dans six sous-populations de deux branches d'une rivière et récolté des données sur l'environnement à chaque site et sur le paysage fluvial. Il existe un gradient positif longitudinal stable sur la période de 8 ans dans la fréquence de tacons mâles matures de l'embouchure à la tête de la rivière. La taille seuil pour la maturation des mâles tacons variait entre les sous-populations; la taille seuil pour la maturation des tacons mâles est plus élevée dans les habitats en aval et plus basse en amont. Ce patron est uniforme dans les deux branches de la rivière durant la période de 8 ans, mais n'est pas en corrélation avec la densité des tacons, ni les caractéristiques physiques des habitats spécifiques à chaque site. Cependant, l'incidence cumulative de caractéristiques de l'habitat pouvant empêcher la migration vers l'amont des grands individus anadromes augmente avec la distance vers l'amont. Les coûts de migration contribuent possiblement aux variations de la taille seuil pour la maturation.

Introduction

The development of alternative phenotypes is often controlled by the state of an individual at a specific moment in its life. The liability trait, which is often a continuous trait such as body size, controls a dichotomous developmental pathway at a threshold node, such that all states above a certain value develop into a given phenotype (Hazel et al. 1990; Roff 1996; Nijhout 2003). In many systems, the threshold has been proposed to be a complex trait that can evolve through natural selection and thus reflect local adaptations (invertebrates: Gotthard 1998; Moczek and Nijhout 2003; Tomkins and Brown 2004; vertebrates: Hutchings and Myers 1994; Nicieza et al. 1994; Hazel et al. 2004).

The dichotomous reproductive tactics of male Atlantic salmon (*Salmo salar*) provide a striking example of two divergent alternative life histories that co-exist within populations. Some males are anadromous, migrating to sea after 2–4 years of residence in their natal river and returning to freshwater to reproduce after 1–3 years at sea. However, other males never leave freshwater and become sexually mature as parr at ages 1 to 3 (Jones and Orton 1940; Hutchings and Jones 1998). These males sneak reproductive opportunities and fertilize a small proportion of the eggs rather than fight with larger and older anadromous males.

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Early maturity occurs in Atlantic salmon male parr if body size exceeds a threshold value during a critical period in the spring before fall reproduction (Saunders et al. 1982; Rowe and Thorpe 1990; Juanes et al. 2000). The incidence of early maturing males is variable among rivers, and variation in the relationship between size at capture and maturation has also been reported among populations (Myers et al. 1986; Hutchings and Myers 1994).

Within a river, the incidence of early male maturity may be expected to covary with the individual growth and condition of fish, such that sites that favour the development of larger and (or) faster-growing males should also favour the development of a higher proportion of early maturing males, as a higher proportion of males would surpass the threshold. This expectation was falsified in a study of male salmon at age 1 (their first opportunity to mature) sampled in different subpopulations of a river. Aubin-Horth and Dodson (2004) observed a poor relationship between size in the spring and the probability of maturing the next fall, indirectly suggesting multiple thresholds for maturation within the same river system. In addition, variation in the relationship between size and maturation incidence observed among tributaries of the same river system and related to altitude was not the result of variation in growth rate (Baum et al. 2004).

Various hypotheses have been formulated to explain variation in the incidence of early maturation of salmon males among different habitats and to relate early maturation to biotic or abiotic characteristics of the environment. For example, the probability of early maturation has been positively correlated with parr density (Baum et al. 2004). Alternatively, the incidence of early maturation has been associated with a certain modal substrate size (Myers et al. 1986). Furthermore, environmental factors may be associated not only with variation in incidence, but also with variation in thresholds in body size for early maturation among subpopulations. For example, Tomkins and Brown (2004) showed that density was the factor driving the evolution of threshold for maturation in two male forms in different populations of European earwig (*Forficula auricularia*) insects.

In this study, we used data acquired from six sample sites in two branches of a river over 8 consecutive years to discriminate the influence of variations in absolute body size and the threshold values in body size for maturation on the incidence of early male maturity in Atlantic salmon. We also examined the influence of environmental characteristics (both the biotic characteristic of parr density and the abiotic characteristics of velocity, depth, and substrate size) in determining variation in early maturation incidence among subpopulations.

Materials and methods

Population studied

The Ste. Marguerite River, Saguenay, Quebec, Canada (48°20′N, 70°00′W), was used as the natural study system. This river has a mean annual discharge of 58 $m^3 \cdot s^{-1}$ and is composed of two distinct branches of approximately equal size, referred to as the principal (PR) and the northeast (NE) branches, which converge 5 km from the outlet located on the Saguenay River. The upstream extent of salmon distribution is limited by impassable waterfalls located 83 km upstream on the principal branch and 32 km upstream on the northeast branch. The northeast branch is characterized by an initial impassable waterfall 7 km upstream, which is bypassed by a fish ladder. The salmon from both branches of the river represent one genetic population, although a significant but weak spatial genetic differentiation occurs among the subpopulations that have been studied (Garant et al. 2000).

Sampling strategy

Our sampling spanned 8 years, from 1996 to 2003. Sampling was performed once a year during the last week of August. We sampled three sites per river branch (six in total; Fig. 1). Sites were separated by 20–30 km within branches. One site (XA01) on the northeast branch was located less than 2 km upstream of another site (NE28) but was within a tributary. In choosing these sites, we took advantage of the prior knowledge that they correspond to previously determined spawning and rearing habitats and that individuals from these sites were weakly but significantly genetically differentiated at neutral markers (Garant et al. 2000). The genetic pattern was consistent among years and therefore these sites were viewed as subpopulations. Salmon offspring usually remain associated with the spawning ground of their parents, which is also suitable nursery habitat, until they migrate to marine feeding grounds. The use of specific parts of the fluvial habitat by juveniles therefore maintains the patchy distribution created by the spawning habitat preferences of their parents. Juveniles at this life stage restrain their movements to less than hundreds of metres, tens of metres in some instances, although a small fraction of older individuals may disperse over a larger area (up to 10 000 m) (Armstrong et al. 1994; Juanes et al. 2000; Webb et al. 2001). These sites also differed in distance from the mouth of the river, thus maximizing the probability of variation in environmental conditions. It has been shown that variation in local conditions linked to upstream location in a river includes width, depth, channel slope, and therefore current velocity, substrate deposition–erosion, and substrate size and arrangement, as well as cover from the banks (Vannote et al. 1980). Indeed, it was already known that the three sites sampled on the principal branch correspond to three sections that exhibit important differences in slope (19.9, 2.7, and 0.8–6.4 $m \cdot km^{-1}$) from upstream to downstream sections. These differences among locations thus signalled potentially major variation in environmental conditions such as temperature, productivity, prey availability, and predation risk (Beisel et al. 1998; Folt et al. 1998; Miyake and Nakano 2002).

Parr maturity incidence

To obtain juvenile individuals, we sampled a distance within each site of between 50 and 100 m in length. This distance incorporates a larger zone than the area covered by the territory of a typical juvenile (Armstrong et al. 1994). Electrofishing was used to sample juvenile Atlantic salmon (parr) at each sampling site. It was not possible to determine age or sex at the time of capture. Individuals were thus sacrificed for laboratory analyses.

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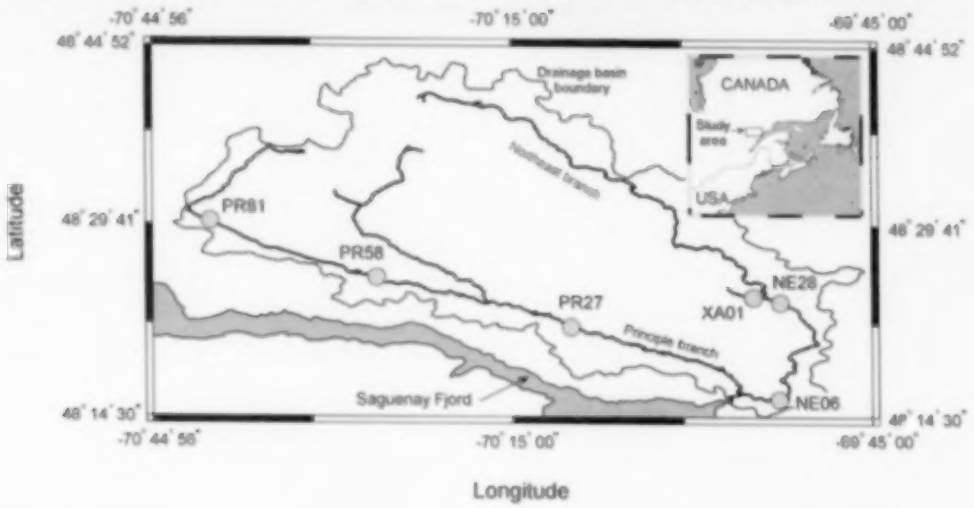
Fig. 1. Location of the six sites sampled from 1996 to 2003 in the Ste. Marguerite River system (Québec, Canada).



Environmental characteristics

Parr densities

Concomitant with the mature parr sampling, an independent ground survey of juvenile Atlantic salmon density was conducted on the principal and the northeast branches of the Ste. Marguerite in August of each year from 1997 to 2004. Between 30 and 48 survey stations were sited along the length of the two branches in each year. In each parcel (5 m by 20 m), juvenile salmon density was estimated using single-pass electrofishing. Juveniles were categorized, on the basis of length, as being either fry (first summer of life) or parr (ages 1–3).

Abiotic environmental characteristics of sample sites

Differences in abiotic environmental characteristics among selected sites were investigated in August 2003. At each site, a rectangular sampling area was defined (320 to 1500 m^2, depending on river width) and divided into a grid of 100 sampling points. At each point, we measured water depth, water surface velocity, and substrate size (using the *b* axis, also known as the intermediate axis, defined as the shortest axis across the maximum projection plane) of a randomly selected pebble.

Airborne survey of the principal branch

Abiotic environmental characteristics throughout the principal branch were determined from an airborne survey, conducted between 1000 and 1500 on 17 and 18 August 2002 using a helicopter-mounted XEOS true-colour digital camera (GENIVAR Inc., Quebec, QC G2J 1C8, Canada). The rationale to using an airborne survey was that it would show how habitat properties varied along the length of the river (the river scape), indicating areas that will impact on migration. Depth and substrate size (D_{50}) were estimated for each pixel using the method outlined by Carbonneau et al. (2004*a*, 2004*b*). River-scape variation in depth and substrate size as a function of distance from the river mouth was determined by estimating the median depth and mean substrate size of each image along the series of images. Images in which more than two-thirds of the channel was less than 30 cm in depth were classified as being those most likely to be subject to low flows; images in which D_{50} was greater than 20 cm were classified as those most likely to contain rapids (fast-flowing water in areas of steep gradients, characterized by the presence of cobbles and boulders).

Analyses

Parr maturity incidence

We determined age group (in years) using the annual patterns present on otoliths (Campana and Jones 1992). We determined sex and maturity state in males by dissection according to the classification of Jones (1940). Mature female parr were never observed. Parr maturity incidence was calculated as the number of males exhibiting ripe testes divided by the total number of males in a sample from a given site. We computed these ratios separately for age-1 and age-2 fish and for each year. We found no age-0 individuals (in their first year of life) to be sexually mature in this system, and older individuals (≥3 years) are very rarely found as almost all migrate to sea at this age (J.J. Dodson, unpublished data). It is important to note that mature male parr that survive can migrate to sea and eventually become large anadromous males (Whalen and Parrish 1999). It was not possible to estimate incidence of early maturation for age-2 males in subpopulation NE06 in 1997 as only one such individual was captured at this site in 1997. Given the binomial nature of the response variable, the incidences of early maturity among male parr were compared using a logit model (Hosmer and Lemeshow 2000). In this model, the effect of branch was taken into account, as well as the effect of sites within branches, the effect of year, the effect of age, and the effect of body size. The model was fitted using the GENMOD procedure of SAS (SAS Institute Inc. 2003). A significant interaction between size and site (within branch) would suggest different threshold sizes for maturation in different subpopulations. The Hosmer–Lemeshow test was

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Table 1. Frequency of early male maturity in each subpopulation for each year and age class, with sample sizes in parentheses.

	Site	1996	1997	1998	1999	2000	2001	2002	2003
Age 1	PR27	0.09 (11)	0.25 (4)	0.29 (34)	0.17 (23)	0.15 (13)	0.08 (13)	0.09 (11)	0.09 (11)
	PR58	0.11 (9)	0.20 (10)	0.47 (15)	0.69 (13)	0.50 (10)	0.22 (9)	0.17 (6)	0.27 (11)
	PR81	0.50 (10)	0.71 (7)	0.75 (8)	0.44 (9)	0.60 (10)	0.18 (11)	0.08 (12)	0.18 (11)
	NE06	0.54 (13)	0.31 (13)	0.50 (32)	0.50 (26)	0.25 (8)	0.00 (7)	0.00 (13)	0.53 (15)
	NE28	0.55 (11)	0.27 (11)	0.30 (10)	0.21 (14)	0.25 (8)	0.28 (18)	0.35 (17)	0.25 (12)
	XA01	0.50 (8)	0.55 (20)	0.73 (11)	0.67 (9)	0.83 (12)	0.38 (13)	0.00 (5)	0.50 (14)
Age 2	PR27	0.50 (12)	0.50 (4)	0.63 (8)	0.75 (4)	0.38 (8)	0.14 (7)	0.13 (8)	0.36 (14)
	PR58	0.78 (9)	0.60 (10)	1.00 (9)	0.88 (17)	0.80 (10)	1.00 (4)	0.70 (10)	0.79 (14)
	PR81	1.00 (9)	0.79 (14)	1.00 (11)	0.85 (13)	1.00 (9)	0.73 (11)	0.70 (10)	0.58 (12)
	NE06	0.55 (11)	*NA*	0.36 (14)	0.71 (7)	0.43 (7)	0.80 (5)	0.17 (6)	0.57 (7)
	NE28	0.75 (4)	0.70 (10)	1.00 (8)	0.75 (8)	0.78 (9)	0.22 (9)	0.63 (8)	0.56 (9)
	XA01	0.80 (10)	1.00 (7)	0.83 (6)	0.75 (20)	0.67 (3)	0.83 (6)	0.62 (13)	0.75 (8)

used to confirm the goodness of fit of the model to the data ($\chi^2 = 5.62$, df = 8, $p = 0.6902$). To quantify the predictive power of the model, the area under the receiver operating characteristics (ROC) curve was calculated ($c = 0.801$). This result was considered as providing excellent discrimination of the two maturity states (mature vs. nonmature). We then employed the "leave-one-out" cross-validation technique to verify the robustness of the model (Stone 1977). We systematically excluded one fish at a time and predicted its maturity state with the logit model adjusted with the remaining data set. We then computed the sensitivity (probability of correctly classifying a fish as mature) and the specificity (correctly classifying a fish as immature) for different probability levels that a fish was mature. The best compromise between sensitivity (66.9%) and specificity (67.0%) occurred at a probability level of 48.8%. Thus, a fish of given size with a predicted probability of being mature exceeding 48.8% was classified as mature. This probability level best discriminated between the two maturity states while minimizing the two errors of misclassification and corresponded to the fraction of mature fish in the total sample. Contrasts were calculated to specify the effect of different factors included in the logit model following the detection of significant sources of variation. Contrasts were conducted using the Fisher protected least significant difference (LSD) method. Additional methods were used to verify the results of multiple comparisons by using the MULTTEST procedure in SAS (SAS Institute Inc. 2003). All tests provided essentially the same results. The relationship between incidence of maturity and distance from sites to the river mouth was established by a decomposition of the sites effect into a linear and quadratic effect of distance.

Threshold body sizes for early maturation

We estimated the threshold body sizes (according to length) for discriminating between mature and immature fish for sites within branches. These threshold body sizes were determined using a probability level of 48.8%, the value that best discriminated between the two maturity states while minimizing errors of misclassification (see above).

Environmental characteristics

Parr densities data were pooled together for upstream and downstream sections of a given river branch and the mean density was compared between upstream and downstream river sections using the Fisher protected LSD method. This was done separately for the principal and the northeast branches of the river. The relationship between mean annual parr density and the estimated incidence of early male maturity was established using Kendall rank correlation.

Univariate analyses of variances (ANOVA) were performed on individual abiotic environmental characteristics to identify whether significant differences in mean properties existed among the sites. For those characteristics exhibiting significant differences among sites, Kendall rank correlations were used to explore their relationship with the estimated incidence of early male maturity.

Results

Variation in parr maturity incidence

The incidence of early maturity was highly variable among years (Table 1). The logit model revealed a significant effect of fish length on the incidence of early male maturity with significant contributions of sites within branches, year, and age (Table 2). After controlling for the effect of size, there was a significant longitudinal gradient in the incidence of early maturing males (Table 2). The incidence of early maturity increased upstream in a linear fashion in both branches of the river (northeast, $p = 0.0003$; principal, $p < 0.0001$). The quadratic function was also significant in the principal branch ($p = 0.0366$), indicating a tendency for the incidence of early maturity to reach an asymptote in this branch (Fig. 2*a*). This positive longitudinal gradient was temporally stable with no year by site (within branches) interaction ($p = 0.27$; Table 2). This stability persisted for almost a decade despite the large variation in the incidence of early maturity among years. Therefore, although the incidence of early maturity changed over time, the ranking according to the incidence of mature male parr within a branch stayed the same, and the gradient from the mouth to the head of the river was conserved. Finally, there was a significant branch by age effect on early male maturity ($p = 0.0096$; Table 2). Two-year-old males from the principal branch showed a significantly higher incidence of maturity than 1-year-old males (65% vs. 39%, respectively, $p = 0.0025$). This was not observed in the northeast branch (48% vs. 46%, respectively, $p = 0.8101$). As a result, the incidence of early male maturity of age-2 males in the principal branch

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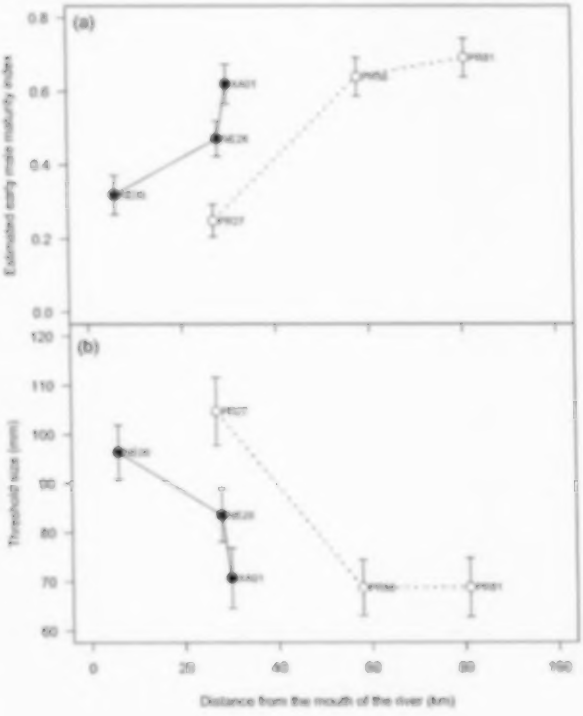
Table 2. Logistic regression (logit function) model using the GENMOD procedure of SAS (SAS Institute Inc. 2003) to establish the relationship between the incidence of early male maturity as a function of body size (length) and the influence of river branch, sampling sites within branches, age of parr, and year of sampling.

Source	df	*F* value	Pr > *F*	χ^2	Pr > χ^2
Length	**1**	**22.8**	**<0.0001**	**22.8**	**<0.0001**
Branch	1	1.21	0.2723	1.21	0.272
Site (branch)	**4**	**16.24**	**<0.0001**	**64.97**	**<0.0001**
Year	**7**	**6.93**	**<0.0001**	**48.54**	**<0.0001**
Year × branch	7	1.05	0.3965	7.33	0.3954
Year × site (branch)	28	1.14	0.2802	31.98	0.2752
Age	1	3.7	0.0547	3.7	0.0543
Branch × age	**1**	**6.7**	**0.0098**	**6.7**	**0.0096**
Site × age (branch)	4	1.36	0.2463	5.44	0.2452
Year × age	7	0.2	0.985	1.41	0.9851
Year × branch × age	7	0.69	0.6769	4.86	0.6769

Note: Significant sources of variation are indicated in bold.

Fig. 2. (*a*) Early male parr maturity frequency among all males and (*b*) threshold sizes (body length at which the probability of maturity is 48.8%) as a function of distance upstream from the river mouth. Solid circles represent the mean of each site of the northeast branch; open circles represent the mean of each site of the principal branch. Vertical bars indicate ±1 standard error.



was greater than that of age-2 males in the northeast branch ($p = 0.0194$).

Variation in threshold body sizes for maturation

Threshold size that a male must reach to mature early varied among the subpopulations sampled. Estimations of the threshold size at which the probability of early maturity equaled 48.8% indicated that the threshold sizes differed significantly among sites in both branches of the river (northeast, $p = 0.0077$; principal, $p < 0.001$; Fig. 2*b*).

Threshold size variation was found to covary with distance from the mouth of the river of each site, and on both branches. The longitudinal gradient in incidence was linked to an interaction between the effect of the subpopulation sampled (site within branch) and size on the probability of maturing (Table 2). In the northeast branch, the threshold level estimated at NE06 was significantly greater than that estimated at the two upstream stations, whereas threshold sizes did not differ between the two most upstream stations (Fig. 2*b*). Similarly, in the principal branch, the threshold level estimated at the downstream station (PR27) was significantly greater than that estimated for the two upstream stations (Fig. 2*b*).

Variation in environmental characteristics

As revealed by a long-term study of juvenile densities conducted in both branches from 1997 to 2004, mean parr density found in the upstream (4.34 parr·m^{-1}, SD = 2.90) and the downstream (3.04 parr·m^{-1}, SD = 2.66) parts of the principal branch did not differ significantly (LSD test, $p = 0.17$). Similarly, juvenile density did not differ between the upstream (4.92 parr·m^{-1}, SD = 3.67) and downstream (4.16 parr·m^{-1}, SD = 3.49) parts of the northeast branch (LSD test, $p = 0.51$). On an annual basis, no significant correlation was found between the predicted incidence of early maturity and the mean annual density in both branches (Kendall rank correlation test, $p = 0.3813$).

Abiotic environmental properties as measured by the ground survey showed a large amount of variation, both within individual sites and between sites (Fig. 3). Comparison by univariate analysis of variance showed that there was a statistically significant difference between the sites in terms of depth ($p < 0.0001$) and velocity ($p = 0.0064$) but not substrate size ($p = 0.3147$). No consistent upstream trend in environmental characteristics existed. For example, median depth at PR58 was greater than that at either the downstream PR27 or the upstream PR81. Kendall rank correlation showed that there was no statistically significant relationship

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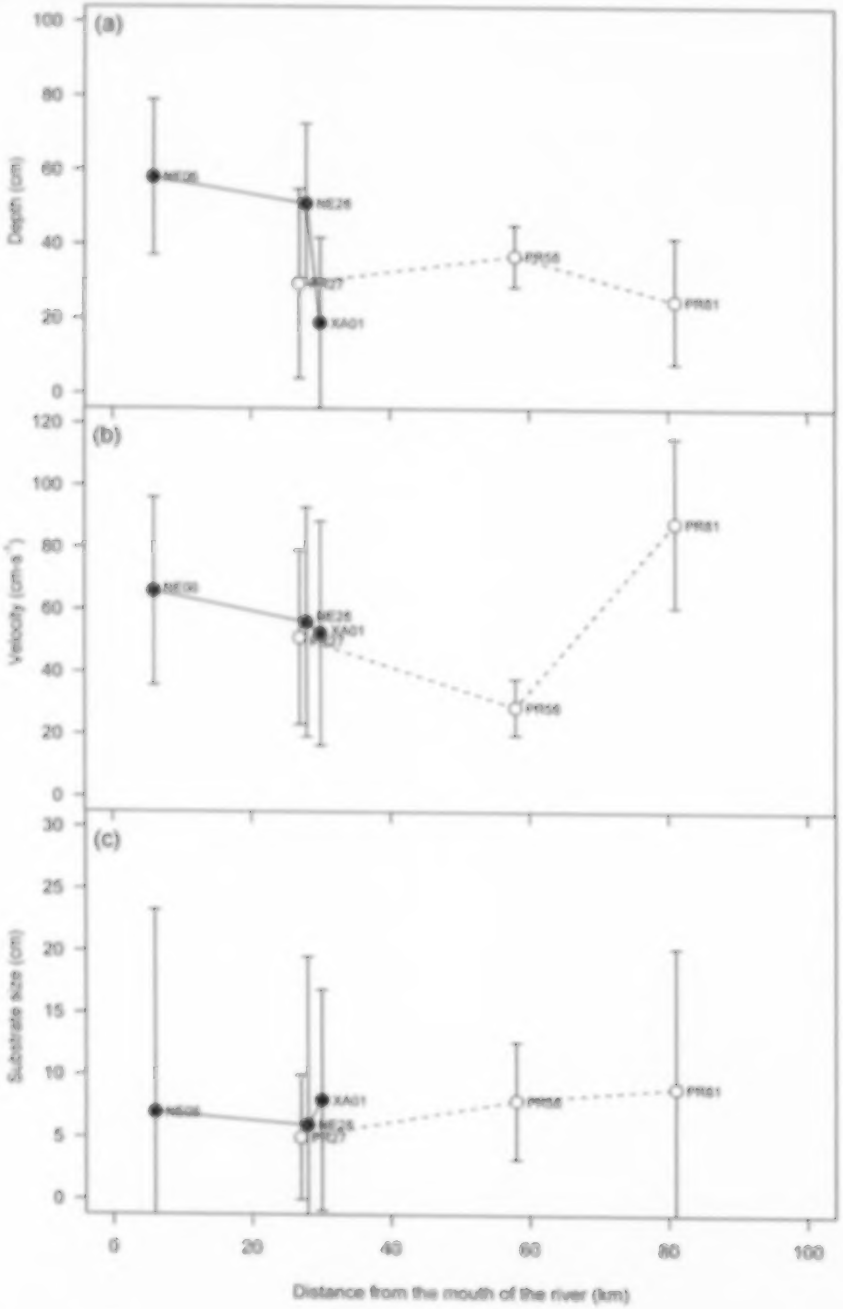
Fig. 3. Habitat characteristics identified from the ground survey as a function of distance upstream from the river mouth: (*a*) depth; (*b*) velocity; (*c*) substrate size. Solid circles represent the median depth, mean velocity, and median substrate size (D_{50}) of each site of the northeast branch; open circles represent the median depth, mean velocity, and median substrate size (D_{50}) of each site of the principal branch. Vertical bars indicate ±1 standard error.



between either depth ($p = 0.4523$) or velocity ($p = 1$) and the estimated incidence of early male maturity.

The airborne survey showed that the cumulative incidence of habitat features that could impede the upstream migration of adult fish, such as sections of the river subject to low flows or rapids, increased with increasing upstream distance (Fig. 4). For example, the incidence of cumulative habitat features that could impede migration was higher upstream

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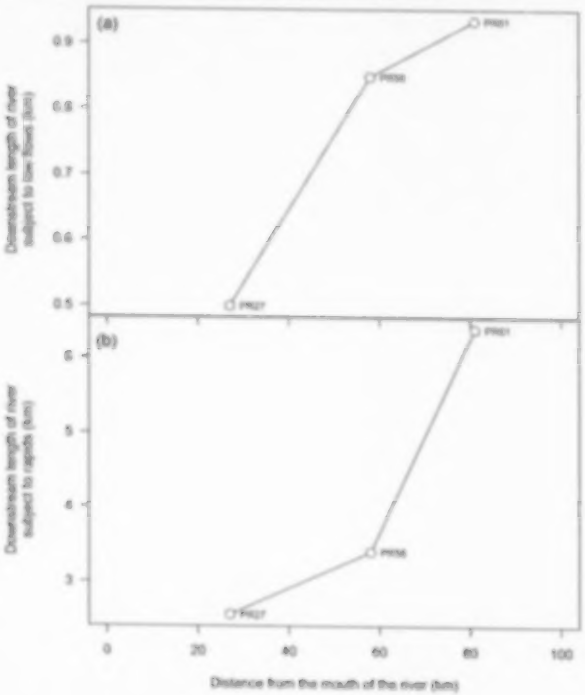
Fig. 4. Cumulative effects of downstream habitat along the principle branch as a function of distance upstream from the river mouth: (*a*) downstream length of river subject to low flows; (*b*) downstream length of river subject to rapids.



on the principal branch, such that a length of river totaling 0.85 km was subject to low flows downstream of PR81, 1.7 times the length downstream of PR 27 (0.5 km); and a length of river totaling 6.40 km was subject to rapids downstream of PR81, 1.9 times the length downstream of PR27 (2.56 km).

Discussion

Variation in body size and in threshold size for maturation have both been proposed to explain among- (Hutchings and Myers 1994) and within-population variation in the incidence of alternative male reproductive phenotypes (Whalen and Parrish 1999; Aubin-Horth and Dodson 2004; Baum et al. 2004). Distinguishing the effects of these two factors has always been a major problem. In the present study, we found that when the effect of size is accounted for, distance from the mouth of the river is a significant predictor of the probability of maturing early in the life history of male salmon (interaction between size and site (within branch) on the probability of maturing). This result is in accordance with the findings by Baum et al. (2004), who found that the altitude of tributaries was a predictor of probability of maturing, independently of size. The spatial pattern found in the present study was temporally stable over almost a decade despite large variation in the absolute proportion of males maturing as parr. In addition, we estimated threshold values for each subpopulation and showed that they varied significantly. Moreover, we showed that this variation in threshold size for development of maturation varied with distance from the mouth of the river, being highest downstream and declining upstream, a pattern found in both branches. Finally, we demonstrated that the observed variation in maturity incidence was not correlated with either parr density or abiotic environmental characteristics measured at the sampling sites.

It is possible that the estimated 20–30 mm shifts in threshold sizes between subpopulations do not translate into any fitness gain or loss. Such an option cannot be ruled out without determining the advantages or disadvantages conferred by developing as a mature parr at a given size in each of the habitats sampled. This possibility can be further examined in future experiments using common rearing environments for offspring of different subpopulations and reciprocal transplants. However, the fact that both branches showed the same trend from the mouth of the river to the most distant part of the system argues against the random variation hypothesis, especially since the trend is clinal in nature, a typical signature of adaptive variation for numerous traits (Endler 1986). Models and empirical data in different species suggest that the trade-offs between costs and benefits associated with expressing a tactic in a given environment influences the threshold that determines whether an individual will express the tactic or not (Hunt and Simmons 2001; Merckx et al. 2003; Hazel et al. 2004). In Atlantic salmon, different environmental and biological variables may modify the fitness that a male of a certain size could achieve using the sneaker tactic. If these fitness-affecting environmental conditions vary with the distance from mouth of the river, it could result in differential selection on threshold values in different subpopulations and the longitudinal pattern observed in this study.

Baum et al. (2004) showed that high parr density was associated with a high incidence of mature male parr. They proposed that higher density leads to smaller size, resulting in lower threshold levels, hence more mature parr. Although competition among mature parr cannot be excluded from playing a role in the pattern of threshold variation observed, our results do not support the Baum et al. (2004) interpretation. No statistically significant relationship was found between parr density and estimated incidence of early maturity.

The observation that the cumulative incidence of habitat features that could impede the upstream migration of adult fish increased with increasing upstream distance suggests that increasing migratory costs associated with the difficulty of upstream migration may favour residency over anadromy by a reduction in early maturity threshold size levels in male Atlantic salmon. In brown trout (*Salmo trutta*), a positive relationship was found between the cost of migration and altitude as revealed by the more rapid decline with altitude in the recruitment of migratory populations than that of resident populations (Bohlin et al. 2001). A positive relationship was also observed between cost of migration and distance in terms of female ovarian investment and male energy reserves and secondary sexual characters in Chinook salmon (*Oncorhynchus tshawytscha*) populations (Kinnison et al. 2001, 2003). We propose that the difficulty of migrating across the river scape is dependent not only on distance, but also on the existence of sections of the river that are shallow or contain rapids: the shallower the depth, the greater the

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probability of interruption to flow during periods of low flows; the greater the prevalence of rapids, the greater the energy required to swim upstream. As a result, males of the upstream subpopulations would be expected to show a higher probability of maturing in fresh water for a given size, thus increasing their probability of reproducing. The energetic costs of migrating over short distances found in this river system are most probably minor. Nonetheless, the cost of upstream migration associated with the increasing probability of losing access to spawning grounds during years of low discharge and (or) high summer temperatures would favour early maturity and lower thresholds upstream, even in the absence of significant energy expenditures. The relationship between the incidence of early male maturity and the difficulty of upstream passage observed here remains anecdotal as it involves only three sites on one river. The relationship needs to be demonstrated in other river systems to adequately test the hypothesis.

In conclusion, the combination of a temporal series and spatial gradients of size and maturation incidence allowed us to determine that variation in incidence among subpopulations of Atlantic salmon was due to differences not only in size, but also in threshold values for maturation. We showed that this variation in threshold size for maturation displayed a longitudinal pattern, being highest downstream and lowest upstream on both branches of the study river. The incidence of parr maturity was not dependent on the biotic or the site-specific abiotic aspects of the environment. However, the incidence of habitat features that could impede migration of large individuals increased with increasing upstream distance. Migration costs may thus contribute to the observed variation in threshold values.

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Consumption and distribution of salmon (*Oncorhynchus* spp.) nutrients and energy by terrestrial flies

Morgan D. Hocking and Thomas E. Reimchen

Abstract: Anadromous Pacific salmon (*Oncorhynchus* spp.) subsidize terrestrial food webs with their nutrients and carcasses, a process driven largely by selective foraging by bears (*Ursus* spp.). We quantify wildlife transfer of salmon carcasses to riparian zones on two watersheds in coastal British Columbia and estimate total terrestrial fly production from remnant carcasses. Large-bodied chum salmon (*Oncorhynchus keta*) were transferred into the forest at a greater rate than were pink salmon (*Oncorhynchus gorbuscha*) (chum salmon mass = 6089–11 031 kg, 16%–48% of salmon run; pink salmon mass = 2266–2808 kg, 4%–6% of salmon run). Blow flies (genus *Calliphora*) and other Diptera dominated colonization (>90% of salmon carcasses). Between the two watersheds, 196 and 265 g of *Calliphora* larvae per metre of spawning length (4 and 7 million larvae for whole watersheds) were generated from salmon carcass transfer. Stable isotope analysis of $\delta^{15}N$ and $\delta^{13}C$ of spring-emerging adult *Calliphora* revealed that >80% of individuals had salmon-based signatures. Flies are a dominant consumer and vector of salmon nutrients in terrestrial habitats and supplement the diet of at least 16 vertebrate and 22 invertebrate species. Anticipated further declines of salmon in the North Pacific can be expected to further erode the complex associations coupling marine and terrestrial ecosystems.

Résumé : Les saumons du Pacifique (*Oncorhynchus* spp.) anadromes enrichissent les réseaux alimentaires terrestres avec leurs nutriments et leurs carcasses, un processus grandement contrôlé par l'alimentation sélective des ours (*Ursus* spp.). Nous mesurons le transfert par l'intermédiaire de la faune sauvage des carcasses de saumons aux zones de rivage dans deux bassins versants de la côte de la Colombie-Britannique et nous estimons la production totale de mouches terrestres dans les carcasses abandonnées. Le transfert des saumons kéta (*Oncorhynchus keta*) plus corpulents se fait à un rythme plus élevé que celui des saumons roses (*Oncorhynchus gorbuscha*) (masse des saumons kéta = 6089–11 031 kg; 16–48 % de la montaison; masse des saumons roses = 2226–2808 kg; 4–6 % de la montaison). Les mouches de la viande (le genre *Calliphora*) et les autres diptères sont les principaux colonisateurs des carcasses (>90 %) de saumons. Dans les deux bassins, respectivement 196 et 265 g de larves de *Calliphora* sont produits par mètre de longueur de frayère (4 et 7 millions de larves pour les bassins entiers) à la suite du transfert des carcasses de saumons. Une analyse des isotopes stables de $\delta^{15}N$ et de $\delta^{13}C$ faite sur les adultes de *Calliphora* qui émergent au printemps montre que >80 % des individus présentent des signatures associées aux saumons. Les mouches sont les plus grands consommateurs de nutriments de saumons et leurs vecteurs principaux vers les habitats terrestres; elles entrent dans le régime alimentaire d'au moins 16 espèces de vertébrés et 22 espèces d'invertébrés à titre de nourriture d'appoint. Les déclins additionnels prévus chez les saumons du Pacifique Nord affaibliront encore plus les associations complexes qui relient les écosystèmes marins et terrestres.

[Traduit par la Rédaction]

Introduction

Every year throughout the North Pacific, millions of anadromous salmon (*Oncorhynchus* spp.) return to their natal watersheds to spawn and die (Cederholm et al. 2000; Gende et al. 2002; Stockner 2003). This reverse nutrient flow, from the sea to land, provides a dominant marine subsidy to aquatic and terrestrial systems and regulates the population dynamics of many wildlife species (Reimchen 1994; Ben-David et al. 1997; Hilderbrand et al. 1999). Historical and continued declines in salmon spawning biomass because of overexploitation, habitat alteration, and climate change threaten salmon-dependent communities coast-wide (Gresh et al. 2000). However, the ecological context of many of these recently described marine–terrestrial associations remains poorly understood.

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Salmon nutrients are distributed from streams and lakes into terrestrial ecosystems mainly through wildlife foraging. Both brown (*Ursus arctos*) and black bears (*Ursus americanus*) consume and distribute salmon carcasses throughout coastal riparian zones of North America and often selectively feed on energy-rich portions of the salmon (Hilderbrand et al. 1999; Reimchen 2000; Gende et al. 2001). The extent of bear predation, carcass consumption, and transfer depends on many factors, the most critical being salmon spawning density, fish size, bear density, and attributes of the habitat that facilitate access (Quinn and Kinnison 1999; Gende and Quinn 2004; Gende et al. 2004*a*).

Salmon carcasses transferred to riparian areas are consumed by many terrestrial scavengers and saprophages. Previous studies have emphasized the dominant role of the Diptera, particularly blow flies (Calliphoridae), in salmon decomposition (Reimchen 1994; Jauquet et al. 2003; Meehan et al. 2005), although total nutrient flow from salmon into this group has not received much attention. At Bag Harbour, Haida Gwaii, ~4000 of 10 690 kg of salmon tissue transferred to the riparian zone by bears were consumed by fly maggots (Reimchen 1994). Large aggregations of dipteran larvae attract an array of insectivores (Cederholm et al. 2000), and the spring emergence of adult Diptera provides a potential food source for numerous consumers.

Herein, we quantify wildlife transfer of salmon carcasses on two watersheds in coastal British Columbia and estimate total terrestrial Diptera production from remnant carcasses. We compare salmon carcass transfer from two separate years to the department of Fisheries and Oceans Canada (DFO) salmon escapement counts and examine variation in rates of predation and transfer between the dominant salmon species *Oncorhynchus keta* (chum salmon) and *Oncorhynchus gorbuscha* (pink salmon). We further estimate the proportion of each transferred carcass consumed by terrestrial Diptera and the number and biomass of Diptera produced in each watershed. We focus on consumption by blow flies (genus *Calliphora*), although we derive estimates for all Diptera using ecological efficiencies of 10% and 20%.

Because salmon have high $\delta^{15}N$ and $\delta^{13}C$ stable isotope signatures relative to most terrestrial sources, stable isotopes can be used to trace the fate of salmon nutrients and energy in terrestrial food chains (Bilby et al. 1996; Ben-David et al. 1998; Hocking and Reimchen 2002). Using stable isotope analysis of $\delta^{15}N$ and $\delta^{13}C$, we assess the frequency of a larval salmon diet in recently emerged adult *Calliphora* as well as the potential for dispersal of flies with salmon signatures to more distant non-salmon habitats.

Materials and methods

Study sites

This study was conducted on two salmon-bearing watersheds, the Clatse (52°20′15″N, 127°50′23″W) and Neekas (52°28′17″N, 128°9′39″W) rivers, on the central coast of British Columbia, Canada. A 5–10 m waterfall truncates spawning at 0.9 km and 2.1 km upstream from the estuary on the Clatse and Neekas rivers, respectively. The climate of this region is cool and wet, with a mean annual temperature of ~8 °C and annual precipitation ranging from 3200 to 4200 mm. Both watersheds occur in the Coastal Western Hemlock biogeoclimatic zone, with forests dominated by western hemlock (*Tsuga heterophylla*), Sitka spruce (*Picea sitchensis*), and western redcedar (*Thuja plicata*) (Green and Klinka 1994; Hocking and Reimchen 2002).

Pink salmon and chum salmon spawn from late August to early November in both watersheds, with black bears and wolves (*Canis lupus*) as the dominant transfer vectors. From field counts, we estimate that 5 and 10 adult black bears were present on the Clatse and Neekas rivers, respectively, in both years. Wolves were present sporadically on the Clatse River in 2001 and on the Neekas River in both years. In 2002, a wolf pack (five adults and seven pups) established a home site in the Clatse estuary. Transfer by wolves was thus more prevalent in 2002 on the Clatse River, although even in this year the majority of salmon carcasses were transferred by bears (>90%). This was determined by the nature of carcass injuries. Wolf predation was defined by the consumption of the whole salmon head with the rest left untouched (Darimont et al. 2003; M. Hocking, unpublished data).

Salmon carcass transfer

In 2001 and 2002, we surveyed the Clatse (15–16 October 2001; 5–6 October 2002) and Neekas (both years: 11–12 October) rivers for salmon carcasses transferred into the forest by vertebrates. This period corresponds to near the end of the salmon run on both watersheds, and very few additional salmon are likely to return.

On the Clatse River, in both 2001 and 2002, 37 parallel belt transects (19 river left, 18 river right) 10 m wide and 100 m in length were used to enumerate pink salmon and chum salmon carcasses transferred into the forest. Transects were separated by 50 m starting from the estuary up to the base of the falls. Owing to a spawning length of 900 m, this method surveyed 20% of the watershed. Each transect was divided into four distance categories from the stream (0–5, 5–20, 20–50, and 50–100 m). Salmon carcasses were identified to species (chum, pink) and placed into one of several consumption categories: (*i*) full carcass; (*ii*) very minor carcass consumption (~95% of fish remains); (*iii*) minor carcass consumption (~80% of fish remains); (*iv*) major carcass consumption (~50% of fish remains); (*v*) nearing complete consumption (~20% of fish remains); and (*vi*) unknown consumption (i.e., skeleton; estimate ~50% of fish was consumed by vertebrate scavenger). Unidentifiable small carcass remnants (~5% of mass or less) were ignored.

On the Neekas River, we used the same transect and carcass classification methodology, except that transects were separated by 100 m rather than 50 m. In 2001, 33 transects (16 river left, 17 river right) were surveyed up to the base of the falls, and because of a spawning length of 2100 m, this method covered roughly 8% of the watershed. In 2002, 41 transects were surveyed (21 river left, 20 river right), with 10% watershed coverage. The 2001 and 2002 surveys differed in that transects in 2002 were extended through the spawning region in the upper estuary.

To estimate the mass of salmon transferred into the forest, full chum salmon and pink salmon carcasses of both sexes were weighed (Table 1). Pink salmon mass did not differ by watershed for either sex (males: $t_{28} = -0.32$, $p = 0.75$; females: $t_{31} = -1.80$, $p = 0.082$), and thus an aggregate mean

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Table 1. Pink salmon (*Oncorhynchus gorbuscha*) and chum salmon (*Oncorhynchus keta*) mass (g) (±1 standard deviation) for both males and females from the Clatse and Neekas rivers, British Columbia.

Watershed	Species	Sex	*n*	Mass (g)
Clatse	Pink	Male	4	1322.5±444.6
		Female	15	971.3±206.4
	Chum	Male	29	4596.6±999.1
		Female	15	3347.3±751.3
Neekas	Pink	Male	26	1384.0±347.2
		Female	18	1124.4±270.8
	Chum	Male	44	3788.6±1377.3
		Female	25	2784.8±810.1

of 1200 g was used. Male and female chum salmon were both larger at Clatse River compared with Neekas River (males: $t_{71} = 2.72$, $p = 0.008$; females: $t_{38} = 2.18$, $p = 0.035$), and thus aggregate means of 3972 and 3287 g were used for chum salmon at the Clatse and Neekas rivers, respectively. This assumes that the sex ratio of spawning salmon transferred into the forest was equal. Since there may be more transfer of males than females owing to their larger size (Reimchen 2000), our carcass mass estimates are likely conservative. Using these aggregate masses, we estimated the mass of salmon remaining after vertebrate foraging for each carcass category (Table 2). An estimate for salmon carcass transfer in the whole watershed was derived by multiplying by 5 on the Clatse River (transect coverage = 20%) and either by 12.7 or 10 on the Neekas River (transect coverage = 8% in 2001 and 10% in 2002). Transfer values were then compared with total salmon escapement determined by DFO (www-heb.pac.dfo-mpo.gc.ca/maps/maps-data_e.htm).

Watershed estimates for salmon transfer and vertebrate consumption were evaluated using two-way analysis of variance (ANOVA), with watershed and salmon species as fixed factors. The variable year (2001, 2002) represented independent replicates for all analyses. The interaction term watershed × salmon species was removed from all tests. Within watersheds, and for each salmon species and year, salmon transfer values (kg·m^{-2}) from forest transects were compared by distance category (0–5, 5–20, 20–50, and 50–100 m) using nonparametric Kruskal–Wallis tests.

Diptera consumption and production

From our carcass surveys on the Clatse and Neekas rivers in 2002, we recorded the percentage of carcasses transferred to the forest that showed signs of dipteran activity, including the presence of adults, larvae, or eggs. Observations only included carcasses transferred to the forest and did not include senescent carcasses flooded on the banks.

We investigated Diptera colonization and consumption of thirty-six experimentally placed carcasses in the fall of 2002 (Clatse $n = 16$; Neekas $n = 20$). We estimated the proportion of each salmon carcass consumed by Diptera and the number of blow fly maggots produced as a function of carcass mass. From 8–10 days after initial carcass placement, final instar *Calliphora* maggots were dispersing from these carcasses. At this time, the volume (mL) of *Calliphora* maggots produced from each carcass was estimated using a 30 mL measuring cup. The volume of other dipteran larvae was visually estimated and likely encompassed the families Dryomyzidae, Heleomyzidae, Muscidae, and Sphaeroceridae. We also reweighed the carcass and determined the change in mass (Δ carcass mass) from initial placement.

To estimate the mass of each individual final instar *Calliphora*, we weighed 30 dispersing larvae from three separate carcasses. *Calliphora* larvae ranged substantially in mass within and between carcasses (overall range: 21.0–118.2 mg; carcass 1: 38.4 ± 11.6 mg (mean ± 1 standard deviation, SD); carcass 2: 49.3 ± 15.3 mg; carcass 3: 85.1 ± 11.2 mg), with an aggregate mean of 57.6 mg. In a separate test, we measured the length and width of 18 *Calliphora* larvae to estimate their volume (volume of cylinder was used), which was compared with measurements of their mass with a paired *t* test. No difference was observed between the two measurements (paired $t_{17} = 1.46$; $p = 0.164$), and thus we used an approximate mass to volume conversion of 1 g = 1 mL. From each carcass, the mass of *Calliphora* produced (g) is thus equal to the mL estimate, and the number of *Calliphora* produced is described by

$$(1)\quad \text{no. of } \textit{Calliphora} = \textit{Calliphora} \text{ volume (mL)} \times \frac{1\,\text{mL}}{0.0576\ \text{g}\cdot\text{individual}^{-1}}$$

To estimate the number and mass of *Calliphora* produced in each watershed from the salmon carcass transfer data, regression equations were used to predict the proportion of the carcass consumed by all Diptera and the number of *Calliphora* produced as a function of Δ carcass mass. Logistic regression was used to predict proportion consumed by Diptera as a function of initial carcass mass ($F_{[1,34]} = 16.34$, $p = 0.0003$, $R^2 = 0.325$):

$$(2)\quad \ln\frac{Y}{1-Y} = -1.96 + 5.29^{-4}X$$

where Y equals proportion consumed by Diptera, and X equals initial carcass mass. Linear regression was then used to estimate the number of *Calliphora* larvae produced by Δ carcass mass. Two regression equations were generated using the minimum and maximum estimates of *Calliphora* volume produced from each carcass (*Calliphora* low (eq. 3*a*): $F_{[1,32]} = 77.7$, $p < 0.0001$, $R^2 = 0.708$; *Calliphora* high (eq. 3*b*): $F_{[1,32]} = 73.2$, $p < 0.0001$, $R^2 = 0.696$):

$$(3a)\quad Y = 224.02 + 2.16X$$

$$(3b)\quad Y = 312.12 + 2.45X$$

where Y equals number of *Calliphora*, and X equals Δ carcass mass. Estimates of mass remaining from carcass categories in carcass transfer data were then used to approximate the number of *Calliphora* produced from carcass transfer on the Clatse and Neekas rivers in 2001 and 2002 (Table 2).

To give an estimate for the average mass of dipteran larvae of all species dispersing from salmon carcasses, we set up a grid of pitfall traps in the fall of 2001 in 10 m × 10 m plots (nine traps per plot) (Hocking and Reimchen 2002). Dipteran larvae varied widely in size, representing the diversity of species and stages consuming salmon. The wet mass of dipteran larvae from nine separate traps on the Neekas ($n = 6$–41 maggots per trap) and seven separate traps on the

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Table 2. Carcass categories (% vertebrate consumption) and number and mass (g) of *Calliphora* (Diptera: Calliphoridae) larvae produced from pink (*Oncorhynchus gorbuscha*) and chum (*Oncorhynchus keta*) salmon carcasses transferred into the riparian zones of the Clatse and Neekas rivers, British Columbia, fall 2001–2002.

% vertebrate consumption	Average mass remaining (g)	Proportion consumed by Diptera	No. of *Calliphora* maggots produced	*Calliphora* mass produced (g)
Pink salmon				
0	1200	0.21	768–929	44.3–53.5
5	1140	0.21	728–883	42.0–50.9
20	960	0.19	618–758	35.6–43.7
50	600	0.16	434–550	25.0–31.7
80	240	0.14	296–393	17.0–22.6
Clatse River chum salmon				
0	3972	0.54	4818–5516	277.5–317.8
5	3773	0.51	4375–5014	252.0–288.8
20	3178	0.43	3181–3662	183.3–211.0
50	1986	0.29	1456–1708	83.9–98.4
80	794	0.18	527–655	30.4–37.8
Neekas River chum salmon				
0	3287	0.45	3384–3892	194.9–224.2
5	3123	0.42	3082–3550	177.5–204.5
20	2630	0.36	2278–2639	131.2–152.0
50	1644	0.25	1117–1324	64.4–76.3
80	657	0.17	460–580	26.5–33.4

Clatse (n = 7–18 maggots per trap) rivers was determined to the nearest 0.1 mg. Mass estimates for each trap were then used to calculate a weighted mean mass of individual dipteran larvae caught in each watershed (Clatse $\bar{X}$ = 21.3 mg; Neekas $\bar{X}$ = 22.9 mg). These masses are lower than the mass estimate for individual *Calliphora* larvae of 57.6 mg.

We estimated the ecological efficiency of *Calliphora* produced from salmon carcasses transferred into the forest by dividing the mass of *Calliphora* produced by the total salmon mass available to invertebrate scavengers for each watershed, year and salmon species. Previous ecological efficiency estimates in the Diptera and other ectotherms have ranged from 7% to 26% (Slobodkin 1960; Putman 1978; Lawton 1981). Using literature values as a guide, we used ecological efficiencies of 10% and 20% to derive estimates of total dipteran larvae production from salmon carcasses that could include all dipteran families on salmon (including Calliphoridae, Dryomyzidae, Spheroceridae, Muscidae, Heleomyzidae, Drosophilidae, and Phoridae). There is often an ecological succession of dipteran species on carrion (Putman 1977), and our empirical observations of *Calliphora* production from salmon carcasses were made early in the decay sequence of the carcass, with an average reduction in mass of 57.9% ± 4.2% and 21.5% ± 11.8% (mean ± standard error, SE) for chum and pink carcasses, respectively. This leaves a substantial mass remaining that is likely partially consumed by more *Calliphora* and other dipteran families. To calculate the number of dipteran larvae produced at these ecological efficiencies, we used the Clatse River mean (21.3 mg) or Neekas River mean (22.9 mg) masses estimated from all dispersing Diptera larvae caught in pitfall traps.

Calliphora and total Diptera production at the watershed level was evaluated using two-way ANOVAs with watershed and salmon species as fixed factors. Upper and lower ranges for *Calliphora* production were averaged, with the variable year (2001, 2002) representing independent replicates for all analyses. The interaction term watershed × salmon species was removed from all tests.

Many predators and parasitoids predate dipteran eggs and instars on the carcass as well as in their dispersing phase (Putman 1977; Ulrich 1999). From the Clatse and Neekas rivers, we enumerated all vertebrate and invertebrate species observed as predators or parasitoids of dipteran larvae on salmon carcasses. This included direct observations of foraging throughout the 2 years of the study and insect collections from the carcasses. Invertebrates were identified to family, vertebrates to species. We also calculated the number and biomass of adult Diptera emerging in the spring. We assumed a pattern of mortality comparable with that of Putman (1977) (66% mortality of larvae dispersing from carcasses and 30% pupal mortality).

Stable isotope analysis

Stable isotope analysis (SIA) of nitrogen (N) and carbon (C) was conducted on adult *Calliphora* collected in baited traps in May and June 2003. Because the tissues of recently emergent insects were expected to reflect their larval diet sources (Tallamy and Pesek 1996), isotope signatures of adult *Calliphora* were used to evaluate the frequency of a larval diet of salmon from both watersheds and to determine if salmon signatures could be found in individuals caught in non-salmon habitats (i.e., above falls). *Calliphora* specimens were caught in hanging traps placed within 10 m of the stream and baited with ~5 g of rotting beef.

Whole *Calliphora* were rinsed with distilled water and then dried at 60 °C for at least 48 h. Samples were ground into a homogeneous powder using a using a Wig-L-Bug

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Table 3. Salmon escapement (Fisheries and Oceans Canada (DFO) counts) and vertebrate consumption and transfer of salmon carcasses from surveys of wildlife-transferred pink (*Oncorhynchus gorbuscha*) and chum (*Oncorhynchus keta*) salmon carcasses on the Clatse and Neekas rivers, British Columbia.

	Clatse River		Neekas River	
	Pink	Chum	Pink	Chum
Salmon escapement (DFO counts)	32 068±9 995	3 150±1 626	57 868±3 016	24 500±7 778
No. of salmon transferred to forest	1 888±1 001	1 533±838	2 340±651	3 356±1 081
Biomass of salmon transferred to forest (kg)	2 266±1 201	6 089±3 329	2 808±781	11 031±3 553
Proportion of salmon run transferred	0.06±0.01	0.48±0.01	0.04±0.01	0.16±0.09
Transferred salmon mass (kg) consumed by vertebrates	897±412	2 969±1 397	1 018±358	4 506±900
Proportion of transferred salmon mass consumed by vertebrates	0.41±0.04	0.50±0.04	0.36±0.03	0.42±0.05
Biomass (kg·m^{-1}) of salmon available for invertebrates*	1.52±0.88	3.47±2.14	0.85±0.20	3.11±1.27

Note: Data represent mean ±1 standard deviation of 2 years: 2001 and 2002.
*Biomass of transferred salmon remnants available for invertebrate consumption per metre of spawning length.

grinder (Crescent Dental Co., Chicago, Illinois). Samples (~1 mg) were assayed for total N, δ^{15}N, total C, and δ^{13}C at the University of Saskatchewan Stable Isotope Facility by continuous-flow isotope ratio mass spectrometry. Natural abundances of ^{15}N and ^{13}C are given in parts per mil (‰) and are expressed by

$$(4) \qquad [(R_{sample}/R_{standard}) - 1] \times 1000$$

where R is the ratio of ^{15}N/^{14}N or ^{13}C/^{12}C. Isotopic standards include N_2 in air for N isotope analyses and Pee-Dee Belemnite limestone for C isotope analysis.

Calliphora δ^{13}C values were lipid-normalized based on equations derived from McConnaughey and McRoy (1979). This was done because there are large C isotope fractionation differences between proteins and lipids (Tieszen and Boutton 1988), and thus this analysis focuses primarily on diet-tissue fractionation from protein rather than lipid metabolism from the various carrion sources to adult *Calliphora*. δ^{15}N and δ^{13}C signatures were compared using two-way ANOVAs with watershed and within-watershed location (above and below waterfalls) as fixed factors.

Isotope signatures were examined relative to possible dietary sources, which include salmon and a variety of terrestrial carrion. The approximate isotopic signatures of pink and chum salmon muscle range from 11‰ to 14‰ for δ^{15}N and –22‰ to –18‰ for δ^{13}C (Welch and Parsons 1993; Kaeriyama et al. 2004), while terrestrial carrion such as deer and small mammals range from –1‰ to 11‰ for δ^{15}N and –30‰ to –21‰ for δ^{13}C (Ben-David et al. 1998; Hocking et al. 2006). DeNiro and Epstein (1978, 1981) observed diet-tissue fractionation values of 1.4‰ to 1.8‰ and –0.6‰ to –0.4‰ for δ^{15}N and δ^{13}C, respectively, in *Calliphora* adults on different diets. For *Calliphora* feeding on a 100% salmon diet, δ^{15}N signatures may be 1‰–3‰ higher than salmon, while δ^{13}C values may be equal to or slightly lower than salmon. As such, we use a cutoff of 12‰ for δ^{15}N and –23‰ for δ^{13}C as our minimum salmon value.

Results

Salmon carcass transfer

The total transfer of chum salmon generally exceeded that of pink salmon, as chum salmon were transferred much more readily relative to their availability in the stream (16%–48% of chum; 4%–6% of pink; $F_{[1,5]} = 12.9$, $p = 0.016$, $R^2 = 0.720$; Table 3). Both pink and chum salmon showed higher escapement on the Neekas River relative to the Clatse River ($F_{[1,5]} = 30.5$, $p = 0.003$, $R^2 = 0.859$), with pink salmon more abundant than chum salmon on both watersheds ($F_{[1,5]} = 53.3$, $p = 0.001$, $R^2 = 0.914$). Both the total salmon mass (kg) and the proportion of available salmon mass consumed by vertebrates was higher for chum salmon compared with pink salmon ($F_{[1,5]} > 7.8$, $p < 0.038$, $R^2 > 0.612$). Of salmon carcasses transferred into the forest, vertebrate consumption varied from 36% to 41% and from 42% to 50% of the available pink and chum salmon mass, respectively, with total consumption ranging from 897 kg for pink salmon on Clatse River to 4506 kg for chum salmon on Neekas River. Averaged across the length of spawning, the mass of salmon remnants in the forest available for invertebrate consumption varied from 0.85 to 1.52 kg·m^{-1} for pink salmon and from 3.11 to 3.47 kg·m^{-1} for chum salmon, depending on watershed.

The biomass (kg·m^{-2}) of pink and chum salmon carcasses transferred to the forest was highest within 5 m of the river edge and decreased with increasing distance from the river in both years and on both watersheds (Fig. 1; all $\chi^2_1 > 8.77$; all $p < 0.001$, except for Clatse River 2001 chum salmon, where $p = 0.032$). Highest transfer of pink salmon occurred on the Clatse River in 2001, while chum salmon transfer was highest in 2002 on both watersheds.

Diptera consumption and production

Calliphora and other Diptera were observed as dominant carcass consumers on virtually all naturally occurring and experimentally placed carcasses on the forest floor. From forest-carcass surveys in 2002, 91.7% of carcasses at Clatse River and 94.7% of carcasses at Neekas River showed signs of dipteran activity, including the presence of adults, eggs, or larvae. Carcasses with no activity were mainly small salmon remnants rather than large carcasses. *Calliphora* colonized 94% of experimentally placed carcasses with 100% of these carcasses colonized by the Diptera as a whole.

Calliphora larvae production from experimentally placed carcasses averaged 311.1 ± 48.3 g·carcass^{-1} (5413 ± 840 individuals) and was strongly predicted by the initial size of the carcass ($F_{[1,34]} = 39.32$, $p < 0.001$, $R^2 = 0.536$). Each *Calliphora* larva consumed an average of 0.70–0.76 g of carcass material (wet mass). This corresponds to an average ecological efficiency of 5.9% ± 2.9% for pink and 7.9% ±

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Fig. 1. Average mass ($kg \cdot m^{-2}$) (± standard error) of (*a*) pink salmon (*Oncorhynchus gorbuscha*) and (*b*) chum salmon (*Oncorhynchus keta*) naturally transferred into the riparian forest of the Clatse and Neekas rivers, British Columbia, in 2001–2002. Measurements are derived from carcass surveys on 5 m × 100 m belt transects perpendicular to the stream and further separated into four distance categories: 0–5 m, solid squares; 5–20 m, open squares; 20–50 m, solid triangles; 50–100 m, open triangles. Numbers below data equal the total number of enumerated carcasses. *n* = number of transects.

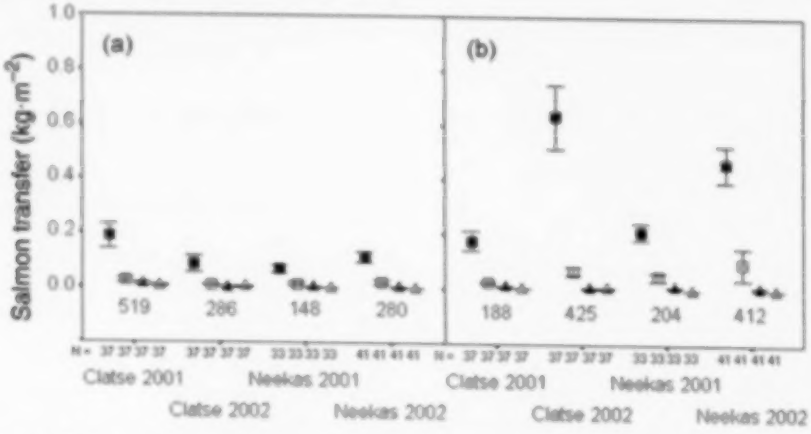
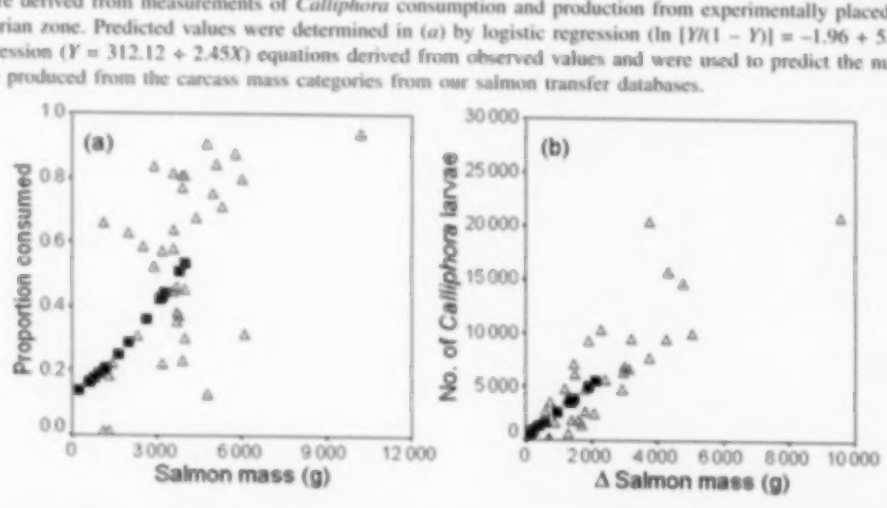


Fig. 2. *Calliphora* (Diptera: Calliphoridae) consumption and production from experimentally placed pink salmon (*Oncorhynchus gorbuscha*) and chum salmon (*Oncorhynchus keta*) carcasses on the Clatse and Neekas rivers, British Columbia. Observed (open triangles) versus predicted (solid squares) results are presented for (*a*) the change in salmon carcass mass (proportion consumed by all Diptera) versus initial salmon carcass mass and (*b*) the number of *Calliphora* larvae produced versus the change in salmon mass. Observed values were derived from measurements of *Calliphora* consumption and production from experimentally placed salmon carcasses in the riparian zone. Predicted values were determined in (*a*) by logistic regression ($\ln [Y/(1 - Y)] = -1.96 + 5.29^{-4}X$) and in (*b*) by linear regression ($Y = 312.12 + 2.45X$) equations derived from observed values and were used to predict the number of *Calliphora* larvae produced from the carcass mass categories from our salmon transfer databases.



1.0% for chum salmon (total range 0%–21%) derived from the initial mass of the carcass. However, when the mass of *Calliphora* produced is divided by Δ carcass mass, this increases to 17.4% for pink (range 0%–26%) and 13.2% for chum (range 0%–28%) salmon.

Excluding the *Calliphora*, dipteran larvae production from remaining fly families on experimental carcasses averaged 53.0 ± 16.6 g for pink and 28.4 ± 4.8 g for chum salmon. Production of these dipteran larvae was negatively predicted by initial carcass mass, as higher productivity occurred on smaller carcasses ($F_{[1,34]} = 8.36$, $p = 0.007$; $R^2 = 0.197$).

The percentage of each salmon carcass consumed by Diptera was predicted by initial carcass mass, while the *Calliphora* production from each carcass was predicted by Δ carcass mass (Fig. 2). Using these predictive equations, we estimated *Calliphora* production from pink and chum carcass categories from our transfer databases in 2001–2002 (Table 2) and then expanded our estimates to include the entire watershed (Table 4). Total *Calliphora* production (in millions of larvae) per spawning season was greater for chum salmon ($3.04–5.75 \times 10^6$) compared with pink salmon ($1.09–1.40 \times 10^6$) ($F_{[1,5]} = 7.8$, $p = 0.038$; $R^2 = 0.610$; Fig. 3). This translates into 38.2–69.8 g of *Calliphora* larvae·m^{-1} of spawning for pink salmon and 157.6–194.6 $g \cdot m^{-1}$ for chum salmon, depending on watershed.

We calculated total dipteran larvae production (number and biomass) per spawning season from both watersheds using ecological efficiencies of 10% and 20% (Table 4). Com-

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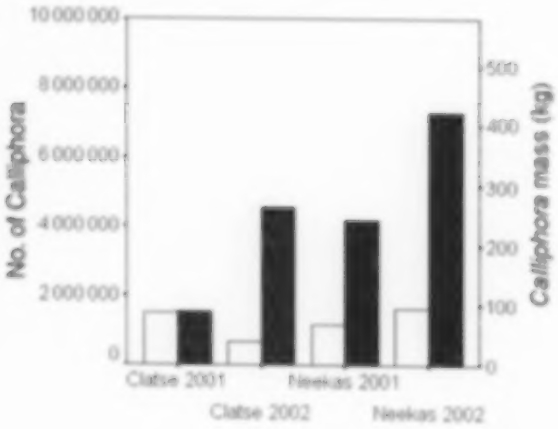
Table 4. Estimates of *Calliphora* (Diptera: Calliphoridae) and total dipteran larvae production and ecological efficiencies from pink (*Oncorhynchus gorbuscha*) and chum (*Oncorhynchus keta*) salmon carcasses transferred to the forest of the Clatse and Neekas rivers, British Columbia.

	Clatse River		Neekas River	
	Pink	Chum	Pink	Chum
No. of *Calliphora* maggots ($\times 10^6$) produced*	1.09±0.60	3.04±2.13	1.40±0.33	5.75±2.20
Biomass of *Calliphora* maggots produced ($g \cdot m^{-1}$)†	69.8±38.6	194.6±136.3	38.2±9.2	157.6±60.2
Ecological efficiency of *Calliphora* on salmon (%)	4.6±0.1	5.5±0.5	4.5±0.04	5.1±0.1
No. of Diptera larvae ($\times 10^6$) at ecoefficiency of 10%*	6.42±3.71	14.64±9.07	7.82±1.85	28.49±11.59
Biomass of Diptera produced at ecoefficiency of 10% ($g \cdot m^{-1}$)†	152.0±87.7	346.5±214.5	85.3±20.2	310.7±126.4
No. of Diptera larvae ($\times 10^6$) at ecoefficiency of 20%*	12.84±7.41	29.28±18.13	15.63±3.69	56.97±23.18
Biomass of Diptera produced at ecoefficiency of 20% ($g \cdot m^{-1}$)†	304.0±175.5	693.0±429.1	170.5±40.3	621.3±252.7

Note: Data represent mean ±1 standard deviation of 2 years: 2001 and 2002.
*Total numbers for each watershed ($\times 10^6$).
†Diptera biomass production in grams per metre of spawning length.

Fig. 3. Estimated number and biomass of *Calliphora* (Diptera: Calliphoridae) larvae produced from carcasses of pink salmon (*Oncorhynchus gorbuscha*) (open bars) and chum salmon (*Oncorhynchus keta*) (solid bars) naturally transferred into the riparian forest of the Clatse and Neekas rivers, British Columbia, in 2001–2002.



bining pink and chum salmon estimates, these values ranged from 396 g of larval Diptera·m^{-1} of spawning at Neekas River to 498.5 $g \cdot m^{-1}$ at Clatse River at an ecological efficiency of 10%. This produces a total of 21 million dipteran larvae at Clatse River and 36 million dipteran larvae at Neekas River (at 10%), with all estimates doubling at an ecological efficiency of 20%.

From Clatse and Neekas rivers, we observed 16 vertebrate and 22 invertebrate species as predators or parasitoids of dipteran eggs or larvae (Tables 5 and 6). Vertebrates included one mammal, two amphibians, and 13 birds. Dominant invertebrates included 13 beetle predators from the Staphylinidae, Silphidae, Hydrophilidae, and Carabidae, and four parasitic wasps from the Ichneumonidae, Braconidae, and Figitidae.

We estimated the biomass and number of adult Diptera emerging in the spring–summer (Table 7). *Calliphora* emergence was greater from chum salmon ($0.70–1.33 \times 10^6$) than pink salmon ($0.25–0.32 \times 10^6$) ($F_{[1,5]} = 7.8$, $p = 0.038$; $R^2 = 0.610$), with production ranging from 8.8–16.2 $g \cdot m^{-1}$ of spawning for pink salmon and 36.5–45.0 $g \cdot m^{-1}$ for chum salmon. Using an ecological efficiency of 10%, overall dipteran adult emergence ranged from 20–80 $g \cdot m^{-1}$ or 1.5–6.6 million adults, depending on watershed and fish species. These values double at an ecological efficiency of 20%.

Table 5. Vertebrate species observed feeding on terrestrial dipteran larvae consuming pink (*Oncorhynchus gorbuscha*) and chum (*Oncorhynchus keta*) salmon carcasses in forest riparian zones of the Clatse and Neekas rivers, British Columbia.

Order	Species
Mammalia	Montane shrew (*Sorex monticolus*)
Amphibia	Rough-skinned newt (*Taricha granulosa*)
	Northwestern salamander (*Ambystoma gracile*)
Aves	Winter wren (*Troglodytes troglodytes*)
	Varied thrush (*Ixoreus naevius*)
	Swainson's thrush (*Catharus ustulatus*)
	Hermit thrush (*Catharus guttatus*)
	American robin (*Turdus migratorius*)
	Song sparrow (*Melospiza melodia*)
	White-throated sparrow (*Zonotrichia albicollis*)
	Golden-crowned sparrow (*Zonotrichia atricapilla*)
	Fox sparrow (*Passerella iliaca*)
	Savannah sparrow (*Passerculus sandwichensis*)
	Steller's jay (*Cyanocitta stelleri*)
	Least sandpiper (*Calidris minutilla*)
	Spotted sandpiper (*Actitis macularia*)

Stable isotope analysis

Stable isotope signatures in spring-emerging adult *Calliphora* varied from 5.0‰ to 19.3‰ for $\delta^{15}N$ and –27.5‰ to –18.4‰ for $\delta^{13}C$, with most values indicating a larval diet of salmon (Fig. 4). Both $\delta^{15}N$ and $\delta^{13}C$ did not vary by watershed or by location above and below the falls (ANOVA both isotopes: all $F < 1.5$, all $p > 0.24$). Means for each watershed ranged from 14.82‰ (Clatse) to 15.12‰ (Neekas) for $\delta^{15}N$ and from –21.94‰ (Neekas) to –21.54‰ (Clatse) for $\delta^{13}C$. Using a cutoff of 12‰ for $\delta^{15}N$ and –23‰ for $\delta^{13}C$, 85.7% of *Calliphora* adults from Clatse River (total $n = 21$) and 81.7% from Neekas River ($n = 60$) were likely raised on salmon carcasses the previous fall.

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Table 6. Invertebrate families observed feeding or ovipositing on terrestrial dipteran larvae from pink (*Oncorhynchus gorbuscha*) and chum (*Oncorhynchus keta*) salmon carcasses in forest riparian zones of the Clatse and Neekas rivers, British Columbia.

Order	Family	No. of species
Coleoptera	Carabidae	4
	Hydrophilidae	1
	Staphylinidae	7
	Silphidae	1
Hymenoptera	Braconidae	2
	Figitidae	1
	Formicidae	1
	Ichneumonidae	1
Araneae	Agelenidae	1
	Antrodiaetidae	1
Parasitiformes	Parasitidae	1
Chilopoda	Geophilidae	1

Discussion

Salmon carcass transfer

Our analysis of salmon carcass transfer to riparian zones of two coastal rivers highlights two principal findings: (*i*) the keystone role of riparian predators, in this case black bears, in the distribution of salmon nutrients and energy to adjacent forest habitats and (*ii*) the increased relative transfer of chum salmon compared with pink salmon.

As salmon return to spawn, bears can predate a large proportion of the available fish, with estimates up to 92% at Bag Harbour, Haida Gwaii (Reimchen 2000), and 100% at the Pedro pond–creek system in Alaska (Quinn and Kinnison 1999). Most observed predation rates, however, are lower, ranging from 2%–70%, and vary by fish species, fish sex, habitat, fish density, and bear density (Gende et al. 2001, 2004*a*; Gende and Quinn 2004). We estimate that from 4% to 6% of the pink salmon run was transferred into the forest, depending on watershed, while chum transfer was higher, ranging from 16% to 48% of the total salmon run. Several studies have observed size-selective mortality in salmon, as bear-killed fish were larger than those dying of senescence (Quinn and Kinnison 1999; Quinn and Buck 2001). Mass-specific energy and mineral composition of spawning chum and pink salmon are roughly equivalent, although chum salmon have much higher absolute values because of their larger size (Gende et al. 2004*b*). Bear transfer of the large-bodied chum salmon has been emphasized in several other studies and can range from 40% to 70% (Reimchen 2000; Gende et al. 2004*b*).

Total salmon transfer declined with distance of placement into the forest. This is comparable with other studies, which have found most salmon transfer to be within 5 m of the stream (Reimchen 2000; Gende et al. 2004*b*). Both Reimchen (2000) and Gende and Quinn (2004) highlight that carcasses with higher mass-specific and absolute energy contents are transferred further into the forest and that one of the main causes of transfer involves conspecific avoidance by subdominant bears. Since energy intake is closely linked to overall fitness, bear foraging likely maximizes energy intake per unit of foraging effort (Gende et al. 2004*a*).

Yearly variation in the rate of salmon transfer was observed in this study, with the most dramatic variation occurring for chum salmon transfer between 2001 and 2002. Yearly variation in transfer is likely linked to yearly variation in salmon escapement and the density of vertebrate predators, as well as environmental factors such as river discharge and flooding (Gende et al. 2001, 2004*a*). In 2002, low precipitation during spawning resulted in low river discharge and higher access to salmon by vertebrate predators and likely contributed to the higher transfer observed in 2002.

Diptera consumption and production

Flies are a widespread consumer and vector of salmon nutrients and energy in terrestrial habitats, with observations from Washington (Cederholm et al. 2000; Jauquet et al. 2003), Vancouver Island (Reimchen et al. 2003), Haida Gwaii (Reimchen 1994), Alaska (Meehan et al. 2005), and now the mainland midcoast of British Columbia (this study). Blow flies (Calliphoridae) typically dominate salmon carrion decomposition, although other families such as Dryomyzidae, Muscidae, Spheroceridae, Phoridae, Heleomyzidae, Trichoceridae, and Sciomyzidae have also been observed. The species *Calliphora terranovae* has been collected from salmon carrion on Vancouver Island (Reimchen et al. 2003), Alaska (Meehan et al. 2005), and the central coast of British Columbia, emphasizing that this species may be particularly adapted to consume salmon carcasses.

From forest-carcass surveys, flies colonized >90% of naturally occurring salmon carcasses in the forest, with uncolonized carcasses mainly including small remnants. Flies also colonized 100% of experimentally placed carcasses, with *Calliphora* colonizing 94% of these. Colonization values are higher than those reported by Meehan et al. (2005), who found that 57% of forest carcasses were utilized by flies, and are closer to estimates by Reimchen (1994), who found that 85% of transferred carcasses were colonized. An important consideration is temperature. Blow flies are sensitive to cold temperatures (Williams and Richardson 1984), and in cooler years they may be out-competed on salmon carcasses by vertebrates and cold-tolerant flies and beetles (Reimchen 1994).

Competition is highly important in structuring species dominance and diversity within carrion communities (Putman 1977; Hanski 1987; Smith and Wall 1997), a process that appears common in communities of invertebrates on salmon carcasses (Hocking et al. 2006). In favourable conditions, *Calliphora* can out-compete most species and can consume entire carcasses within several days (Meehan et al. 2005). We observed high *Calliphora* production on large carcasses and more production of other dipteran larvae on smaller carcasses. Flies have been shown to partition themselves by carcass size (Kneidel 1984; Hanski 1987), and variable consumption of salmon by bears and variable fish sizes may increase the taxonomic diversity of insect species on salmon.

Another niche dimension along which carrion species are often distributed is the successional stage of the carcass itself (Putman 1977; Anderson and VanLaerhoven 1996). Our

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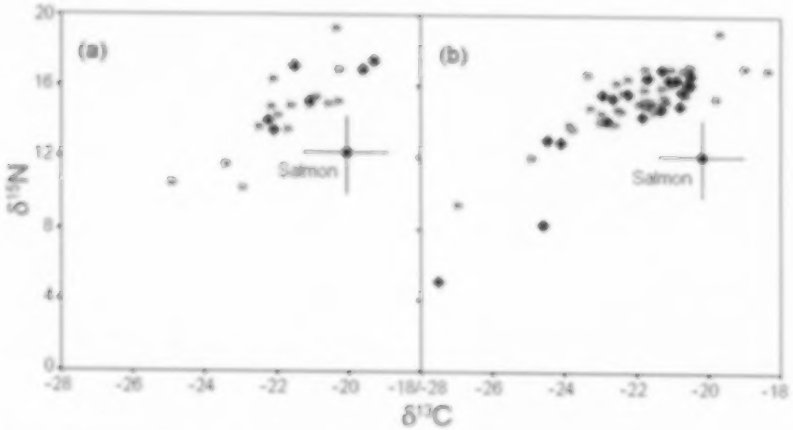
Table 7. Estimates of *Calliphora* (Diptera: Calliphoridae) and total dipteran adult spring–summer emergence from pink (*Oncorhynchus gorbuscha*) and chum (*Oncorhynchus keta*) salmon carcasses transferred to the forest of the Clatse and Neekas rivers, British Columbia.

	Clatse River		Neekas River	
	Pink	Chum	Pink	Chum
No. of emerging *Calliphora* adults ($\times 10^6$)[a]	0.25±0.14	0.70±0.49	0.32±0.08	1.33±0.51
Biomass of emerging *Calliphora* adults ($g \cdot m^{-1}$)[b]	16.2±8.9	45.0±31.5	8.8±2.1	36.5±13.9
No. of emerging Diptera ($\times 10^6$) at ecoefficiency of 10%[a]	1.49±0.86	3.38±2.09	1.81±0.43	6.58±2.67
Biomass of emerging Diptera at ecoefficiency of 10% ($g \cdot m^{-1}$)[b]	35.1±20.2	80.1±49.7	19.7±4.7	71.8±29.2
No. of emerging Diptera ($\times 10^6$) at ecoefficiency of 20%[a]	2.97±1.71	6.76±4.19	3.61±0.86	13.16±5.35
Biomass of emerging Diptera at ecoefficiency of 20% ($g \cdot m^{-1}$)[b]	70.3±40.5	160.1±99.1	39.4±9.3	143.5±58.4

Note: Data represent mean ±1 standard deviation of 2 years: 2001 and 2002.
[a]Total numbers for each watershed ($\times 10^6$).
[b]Diptera biomass production in grams per metre of spawning length.

Fig. 4. $\delta^{15}N$ and $\delta^{13}C$ stable isotope signatures in spring-emergent *Calliphora* (Diptera: Calliphoridae) adults from above (solid diamonds) and below (asterisks) the falls on the (*a*) Clatse and (*b*) Neekas rivers, British Columbia. *Calliphora* were caught in baited traps and were assumed derived from salmon carrion if isotope signatures are higher than 12‰ for $\delta^{15}N$ and –23‰ for $\delta^{13}C$ (solid circle, Salmon).



measurements of *Calliphora* production from salmon carcasses occurred fairly early in the decay sequence, with often a relatively large mass remaining for further consumption. If other Diptera families are more competitive on smaller carcasses, then they may be more prevalent in the later decay stages of larger carcasses.

Of other studies that have emphasized Diptera consumption of salmon carcasses, only Reimchen (1994) and Meehan et al. (2005) attempt to quantify fly maggot production at a watershed level. We found that 4.13 million and 7.15 million *Calliphora* larvae were produced on the Clatse and Neekas rivers, respectively, from salmon carcasses transferred to upland areas per spawning season. These values are lower than Meehan et al. (2005) in terms of overall production (i.e., 10 million larvae), likely because of lower estimates of production per carcass. However, estimates for *Calliphora* production were derived from conservative ecological efficiencies of 4.5%–4.6% for pink and 5.1%–5.5% for chum salmon. If we include other fly families using ecological efficiencies of 10% and 20%, then our estimates for total Diptera production from salmon carcasses becomes higher.

Larval Diptera are important vectors of marine nutrients and energy to numerous vertebrate and invertebrate consumers in terrestrial food webs. Our observation of 16 vertebrate and 22 invertebrate predators or parasitoids associated with dipteran larvae on salmon carcasses expands on previous observations by Reimchen (1994), Cederholm et al. (2000), and Jauquet et al. (2003) in British Columbia and Washington. Overall, the extent to which these species depend on larval flies derived from salmon carcasses for life history attributes, including reproduction, migration, and overwintering, is relatively unknown. It is possible, for example, that the parasitic wasps observed here have a population life history that is timed to seasonal cycle of salmon, as has been documented for coastal and riverine mink (*Mustela vison*) (Ben-David et al. 1997).

Across all known chum and pink salmon spawning watersheds on the central and north coasts of British Columbia ($n > 500$), total escapement biomass over the last 50 years has averaged roughly 6.5 and 10 million kg for chum and pink salmon, respectively (DFO data available from www-heb.pac.dfo-mpo.gc.ca/maps/maps-data_e.htm). Much of this biomass is either consumed by multiple terrestrial vertebrates or ends up in the form of senescent carcasses that are consumed within aquatic food chains (Bilby et al. 1996; Wipfli et al. 1998). The remaining mass transferred to riparian forests and not consumed by vertebrates is made available for invertebrate scavengers, primarily including the

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Diptera. More transfer, less tissue consumption, and consequently more Diptera production is expected when salmon densities are high and salmon are more accessible. Given that the Clatse and Neekas are both small rivers that provide good access to salmon, our estimates of transfer and Diptera consumption may be slightly higher than an average coastal chum and pink salmon run. However, extensive Diptera production from salmon carcasses may also occur in systems supporting sockeye salmon (*Oncorhynchus nerka*), as numerous studies have documented bear predation on sockeye (Quinn and Kinnison 1999; Gende et al. 2001; Quinn and Buck 2001) and Diptera colonization of their carcasses (Meehan et al. 2005).

Stable isotope analysis

Emerging *Calliphora* adults caught in baited traps in the spring indicated a high frequency (>80%) of a larval diet of salmon from both the Clatse and Neekas rivers. The mean isotope signature of both δ^{15}N and δ^{13}C did not differ by location of collection within each watershed, as high values were also present in individuals collected above the falls. This highlights the further distribution of salmon nutrients to non-salmon habitats in the form of dispersing flies. Riparian corridors are known to be important locations for movement of many animals, including flies (Fried et al. 2005), and emerging *Calliphora* could easily disperse to above the falls in search of mates and carcasses for breeding.

Implications

Spawning salmon provide a dominant source of nutrients and energy to hundreds of aquatic and terrestrial species throughout the North Pacific (Cederholm et al. 2000; Stockner 2003). Salmon have been labelled as "ecosystem engineers" (Schindler et al. 2003) and "intrinsic to ecosystem function" (Zhang et al. 2003) and provide a cross-habitat subsidy that may be thought of as similar in magnitude to the annual migrations of wildebeest (*Connochaetes* spp.) on the Serengeti (Reimchen 2000). We document and quantify a largely unreported yet critical component of this process — the consumption and distribution of salmon-derived nutrients and energy by terrestrial flies. Despite higher escapement of pink salmon, large-bodied chum salmon were preferred prey for bears and were transferred more readily into the forest. The main predictors of total Diptera consumption were linked with carcass size and availability, which is correspondingly linked to the density of fish spawning, the species of fish (chum or pink), bear density, and attributes of the habitat (Reimchen 2000; Gende et al. 2001). Overall this means that the most Diptera production will occur in situations where there is high density spawning of chum salmon, large numbers of bears to transfer carcasses, and streams that facilitate access and selective foraging by vertebrate predators. The presence of flies breeding on salmon carcasses may thus be used as an indicator for not only the presence of bears or other wildlife but adequate salmon escapement to support both vertebrate and invertebrate scavengers.

The ecological implications of this process of salmon transfer by vertebrates and subsequent consumption of carcasses by terrestrial Diptera is most relevant when considering the coast-wide declines in salmon spawning biomass over the last century (Finney et al. 2000; Gresh et al. 2000). Throughout the coast of British Columbia, it is small chum salmon streams that are experiencing recent declines (Godbout et al. 2004; Spilsted 2004), precisely the watersheds that may be the most critical for preserving the salmon–bear–Diptera linkage. The consumption of salmon carcasses by terrestrial Diptera results in the widespread distribution of salmon nutrients and energy to multiple species, with the potential increases in biodiversity and carrying capacity of these coastal habitats.

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Diatom reference communities in Québec (Canada) streams based on Kohonen self-organizing maps and multivariate analyses

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Abstract: The identification of biological reference conditions specific to each type of water body is essential for the development of sound biological indicators and criteria. The purpose of the present study was to establish the reference conditions of each stream type sampled in southern Québec (Canada) using benthic diatoms and environmental variables characterizing streams and watersheds. First, stream reaches were classified as a function of their natural watershed and habitat characteristics. Second, diatom communities were classified based solely on taxa abundance data. Resulting groups were graphically presented on ordinations to interpret, a posteriori, the environmental gradients associated with diatom groups and to identify the diatom communities representing the reference conditions of each of the stream reach groups. A final classification based solely on diatom reference communities found pH and conductivity to be the main discriminating factors, regardless of ecoregion and stream type. Although a specific diatom reference community may be identified for each stream group, our results suggest that many of these communities exhibit strong similarities. Only two reference communities may therefore be used, one for circumneutral conditions and one for alkaline conditions. These reference communities represent the baseline for biocriteria development.

Résumé : L'identification des conditions de référence biologiques spécifiques à chaque type de masse d'eau est essentielle pour le développement de bioindicateurs et de critères biologiques rigoureux. L'objectif de cette étude est d'identifier les conditions de référence spécifiques à chaque type de cours d'eau à partir des communautés de diatomées benthiques et des variables environnementales caractérisant les rivières et les bassins versants du Québec méridional (Canada). Dans un premier temps, les tronçons de rivières ont été classifiés en fonction des caractéristiques naturelles des bassins versants et des habitats lotiques. Dans un deuxième temps, les communautés de diatomées ont été classifiées d'après uniquement les données d'abondance, sans recours aux variables environnementales. Les groupes qui en résultent ont été représentés graphiquement sur des ordinations afin d'être en mesure d'interpréter, a posteriori, les gradients environnementaux influençant les diatomées et d'identifier les communautés qui représentent les conditions de référence de chacun des groupes de rivières. Finalement, une classification basée uniquement sur les communautés de référence a démontré que le pH et la conductivité sont les facteurs les plus discriminants, quels que soient l'écorégion et le type de cours d'eau. Bien qu'une communauté de référence puisse être identifiée pour chaque groupe de tronçons, nos résultats suggèrent que plusieurs de ces communautés sont similaires. Seulement deux communautés de référence ont par conséquent été établies, une pour les conditions circumneutres et l'autre pour les conditions alcalines. Ces communautés peuvent être considérées comme des références pour l'établissement de biocritères.

Introduction

The evaluation of aquatic ecosystems health should be realised by comparing the observed ecological conditions with the expected conditions, in the absence of human disturbance. These reference conditions can be derived from historical data (e.g., Nijboer et al. 2004), regional reference sites (e.g., Gosselain et al. 2005), prediction models (e.g., Wright et al. 1998), paleolimnological data (e.g., Simpson et al. 2005), or expert judgement. According to the US Environmental Protection Agency (Gibson et al. 1996) and the European Water Framework Directive (European Parliament

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2000), regional reference sites provide the most realistic basis and are the most common approach used for the establishment of reference conditions. The use of a regional-based reference is preferred over individual site-specific references, because it allows for a broader application in water resource programs.

Ecoregions are usually the preferred classification scheme for establishing regional site classes. The ecoregion concept recognizes geographic patterns of similarity among ecosystems, grouped on the basis of environmental variables such as climate, soil type, physiography, and vegetation (Omernik 1987). However, the ecoregion scheme may be refined to define biological potential and identify reference conditions within ecoregions and subecoregions. A classification based on stream attributes may be useful to provide an ecological basis for identifying homogeneous areas from which reference conditions can be established.

Contrary to the regional reference site approach, prediction models usually make no a priori assumptions about the similarity of biological communities at different sites. The reference sites are classified using clustering methods based solely on the similarity of their species composition (Reynoldson et al. 1997). A test site is then matched to the appropriate reference group using, for example, a discriminant function analysis. Such models were used for the development of bioassessment tools such as RIVPACS (river invertebrate prediction and classification system; Wright et al. 1993), AusRivAS (Australian river assessment scheme; Parsons and Norris 1996), and BEAST (benthic assessment of sediment; Reynoldson et al. 1995).

The reference condition approach is being increasingly integrated into many bioassessment programs and is entrenched in the regulatory structure of environmental policies, such as the Clean Water Act (CWA) in the USA and the European Water Framework Directive (WFD). In USA, the need to identify reference conditions has led to a number of studies concerning periphyton communities (e.g., Pan et al. 2000), benthic invertebrate fauna (e.g., Barbour et al. 1999), and fish fauna (e.g., Baker et al. 2005). In Europe, the WFD has initiated a number of studies establishing the reference conditions for phytoplankton (e.g., Lepisto et al. 2004), macrophytes (e.g., Schaumburg et al. 2004*a*, 2004*b*; Meilinger et al. 2005), phytobenthos (e.g., Foerster et al. 2004; Gosselain et al. 2005; Tison et al. 2005), benthic invertebrate fauna (e.g., Rawer-Jost et al. 2004), and fish fauna (e.g., Oberdorff et al. 2001; Carrel 2002).

In Canada, benthic macroinvertebrates are the most widely used group of aquatic organisms in bioassessment. The reference condition approach has mainly been used in Ontario (e.g., Reynoldson et al. 1995; Linke et al. 1999; Winter et al. 2003), British Columbia (e.g., Reynoldson et al. 1997, 2001), and the Yukon (Bailey et al. 1998). The reference condition approach has also been used for fish communities, although to a lesser extent (e.g., Tonn et al. 2003). In the case of algal communities, extensive work has been conducted in Canada regarding the reference conditions of lake diatom assemblages derived from paleolimnological investigations (e.g., Smol et al. 1998). However, for lotic ecosystems, few studies have used diatoms and other periphytic algae as indicators of ecological integrity. Most of these studies were conducted in Ontario (e.g., Winter and Duthie 2000*a*, 2000*b*; Winter et al. 2003) and Québec (Wunsam et al. 2002; Campeau et al. 2005; Lavoie et al. 2006).

The purpose of the present study was to establish the regional reference conditions of each stream type sampled in southern Québec (Canada) using benthic diatoms and environmental variables characterizing streams and watersheds. First, stream reaches were classified as a function of their natural watershed and habitat characteristics using Kohonen self-organizing maps (SOM). Second, diatom communities were classified based solely on taxa abundance data. Resulting groups were graphically presented on ordinations to interpret, a posteriori, the environmental gradients associated with diatom groups and to identify the diatom communities representing the reference conditions of each of the stream reach groups. These reference communities represent the baseline for biocriteria development. They may also be considered as target communities in the context of stream and river restoration.

Material and methods

Study area

The study area is distributed within three ecoregions: the Canadian Shield, the St. Lawrence Lowlands, and the Appalachians (Fig. 1). These ecoregions were further subdivided into natural provinces by Li and Ducruc (2000). The southern part of the Canadian Shield is underlain by acidic igneous and metamorphic rocks (granite, gneiss, migmatite, etc.) covered by noncalcareous glacial tills low in clay-sized particles (Vincent 1989). The Canadian Shield contains an intricate hydrological network of lakes, peat bogs, marshes, beaver ponds, rivers, and streams. Its catchments are mostly covered by boreal forest with humo-ferric podzol soils (Clayton et al. 1978). The southern part of the Shield, however, overlaps the transition zone of mixed and boreal coniferous forests. The streams sampled in the Canadian Shield were low in nutrients, conductivity, and suspended solids and exhibited circumneutral pH (Table 1). These catchments are considered to be the least disturbed. However, some lake outlets may occasionally have higher nutrient levels.

The St. Lawrence Lowlands is a low-lying region with Paleozoic carbonate bedrock, overlain by glacial sediments and postglacial marine clays, and characterized by fertile soils. The lowlands encompass three natural provinces, which differ in geology, geomorphology, and land use (Fig. 1). The St. Lawrence Lowlands is the most heavily populated area of Québec and is characterized by intensive farmlands and large industrial centers. The streams sampled in this ecoregion were high in nutrients, conductivity, suspended solids, and pH (Table 1). These catchments exhibited a gradient from slightly impacted to very impacted streams with most of the latter being located in the Upper St. Lawrence Plain.

Located in southeastern Canada, the Appalachian Mountains are a geologically complex region with low and rounded relief. The rocks of this range are sedimentary, dating back to the Paleozoic era, and are covered by glacial tills. This region is also impacted by farming, but to a lesser extent. The streams sampled in this region had intermediate levels of nutrients, conductivity, and suspended solids. The Appalachian Complex of the Lower St. Lawrence is one of two natural provinces in the Appalachian region and exhibits

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Fig. 1. Sampling locations in the St. Lawrence River basin (Québec, Canada). The Appalachians and the St. Lawrence Lowlands were subdivided into natural regions following Li and Ducruc (2000).

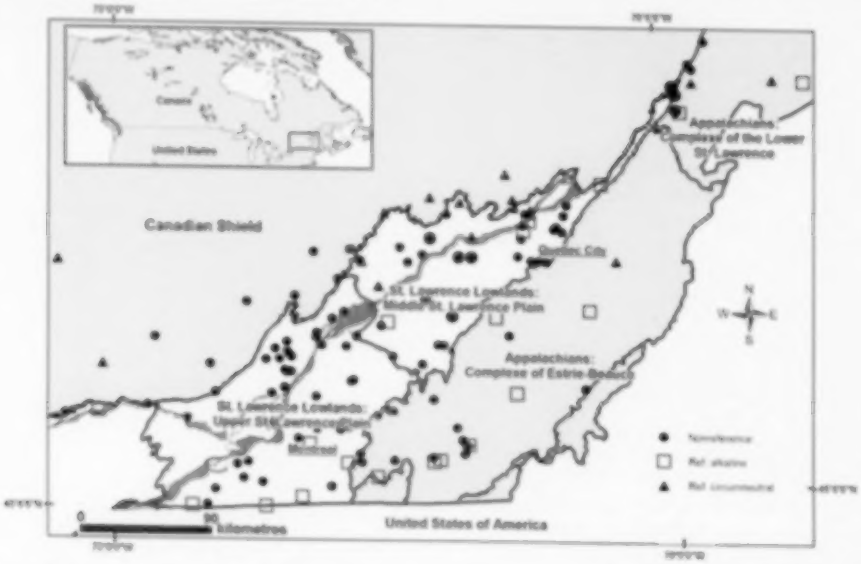

Table 1. Median values (M) and first and third quartiles (Q1 and Q3, respectively) for water chemistry variables arranged according to ecoregions of the St. Lawrence River basin (Québec, Canada).

Code	Description		Canadian Shield			St. Lawrence Lowlands			Appalachians		
			Q1	M	Q3	Q1	M	Q3	Q1	M	Q3
TP	Total phosphorus (mg·L^{-1} P)	Log	0.015	0.017	0.020	0.026	0.051	0.131	0.022	0.024	0.036
SRP	Soluble phosphorus (mg·L^{-1} P)	Log	0.010	0.010	0.011	0.011	0.023	0.057	0.010	0.010	0.012
TN	Total nitrogen (mg·L^{-1} N)	Log	0.182	0.210	0.258	0.330	0.633	1.353	0.275	0.410	0.560
NO_3	Nitrates-nitrites (mg·L^{-1} N)	Log	0.038	0.052	0.083	0.126	0.315	0.877	0.095	0.161	0.303
NH_3	Ammonia (mg·L^{-1} N)	Log	0.020	0.020	0.025	0.025	0.036	0.057	0.022	0.025	0.050
CHL	Chlorophyll *a* (mg·m^{-3})	Log	1.9	2.5	2.8	3.4	7.3	13.9	2.8	4.2	7.9
PH	pH	—	7.1	7.3	7.3	7.8	8.1	8.4	7.8	8.0	8.2
CON	Conductivity (μS·cm^{-1})	SQR2	29	38	70	160	273	393	136	163	233
T	Water temperature (°C)	—	19.2	20.2	20.9	19.7	21.5	22.5	19.8	21.2	22.2
O_2	Dissolved oxygen (mg·L^{-1})	—	8.7	9.1	9.7	8.2	9.1	10.0	8.7	9.2	9.9
TUR	Turbidity (NTU)	Log	0.8	1.3	2.0	2.5	5.6	10.7	1.4	2.7	4.1
SS	Suspended solids (mg·L^{-1})	Log	2.0	2.4	3.4	4.2	7.3	16.4	2.3	3.3	5.5
FC	Coliforms (UFC·100·mL^{-1})	Log	23	44	86	122	277	1024	56	123	215
DOC	Dissolved organic carbon (mg·L^{-1})	Log	4.0	4.6	5.2	4.7	6.0	7.6	3.9	5.7	6.7

Note: Log, log-transformed; SQR2, square root transformed; NTU, nephelometric turbidity units.

the highest concentrations of dissolved organic carbon due to the presence of wetlands in some of its catchments.

Many streams originate in the Canadian Shield or the Appalachians and flow downstream through the St. Lawrence Lowlands. As a result, the water chemistry of some streams flowing through the lowlands reflects the characteristics of upstream ecoregions. This is especially true for the large rivers of the northern shore flowing through the middle St. Lawrence Plain and the Ottawa Plain.

To account for interannual variability within diatom communities, sampling was conducted in the fall (September) of 2002 and 2003. These samples were collected at 126 sampling locations distributed along 32 rivers and streams in the St. Lawrence River basin. A total of 204 diatom samples were collected and analysed, 111 samples in 2002 (coded as "B") and 93 in 2003 (coded as "D"). The sampling locations are part of the water quality monitoring network of the Québec Ministry of the Environment. These sites were selected according to the availability of physico-chemical data and on the basis of land use information with the aim of sampling across a broad gradient of ecoregions and pollution levels. In 2003, the Québec Ministry of the Environment removed a number of sites from its monitoring network, and although new sites were added, the result was a lower number of sites sampled in 2003.

Water chemistry

Water analyses were performed by the Ministry of the En-

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vironment (Québec Government) as part of a water quality monitoring program that began in the 1970s. Most water samples were collected every 4 weeks. The following parameters were considered in this study: total phosphorus (TP), soluble reactive phosphorus (SRP), total nitrogen (TN), nitrate/nitrite nitrogen (NO_3), ammonia nitrogen (NH_3), chlorophyll *a* (CHL), pH, conductivity (CON), temperature (T), dissolved oxygen (O_2), turbidity (TUR), suspended solids (SS), faecal coliforms (FC), and dissolved organic carbon (DOC). Some water chemistry data were transformed to improve normality (Table 1). Because diatoms are known to integrate stream water chemistry through time, seasonal averages were used in the analyses. Averages of stream water chemistry were calculated from the six measurements taken in August and September over a 3-year period, including the year in which the diatoms were sampled. These 3-year seasonal averages explain more variance in diatom community structure than one-time chemistry measurements (Campeau *et al.* 2005).

Stream habitat and watershed characteristics

Stream reach embankment, width, and morphology, current velocity, water transparency, water level, substrate type, and riparian zone characteristics were evaluated at each site (Appendix A). A geographic information system (ArcGIS, version 8; ESRI, 1999) was used to determine watershed characteristics upstream of each sampling sites, such as watershed area, distance to source, geology, surficial deposits, land use, cropped area, animal units, population, and ecoregions (Appendix A). The following data were made available by several governmental agencies: digital maps were provided by the Québec Ministry of the Environment, geologic maps were provided by the Québec Ministry of Natural Resources, classified Landsat images were supplied by the Québec Ministry of Agriculture and Fisheries, and census data were contributed by Statistics Canada. Supplementary information from the United States Geological Survey (USGS) was used for the characterisation of watersheds located beyond the Canadian border.

Diatom data

Benthic diatoms were scraped from the top surface of five rocks (composite sample) from riffles in unshaded areas where possible. Samples were collected within a ~5 m^2 area at depths varying from 20 cm to 50 cm, depending on water level and turbidity. The samples were preserved with Lugol's iodine and stored until they were processed. The samples were digested using hydrogen peroxide and mounted onto microscope slides using Naphrax. A minimum of 400 valves (Prygiel and Coste 1993) per slide were counted and identified at 1250× to the most precise possible taxonomic level under a Zeiss Axioskop II microscope with differential interference contrast imaging (DIC). Taxonomic identifications generally followed Krammer and Lange-Bertalot (1986, 1988, 1991*a*, 1991*b*), Reavie and Smol (1998), Fallu et al. (2000), Krammer (2000, 2002, 2003), and Lange-Bertalot (2001).

Stream reach classification and identification of reference diatom communities

Diatom communities in natural environments are primarily influenced by water physico-chemistry (e.g., pH, CON, DOC) reflecting watershed attributes, such as geology, surficial deposits, and wetlands. Habitat characteristics, including substrate, shade, and current velocity, also play an important role in the structuring of diatom communities. Stream reaches in their natural conditions and with similar habitat and watershed characteristics should, therefore, have comparable benthic diatom communities. The first step in the determination of reference diatom communities was to classify stream reaches according to their habitat and watershed characteristics, excluding variables influenced by anthropogenic factors (Fig. 2). The second step consisted of the classification of diatom communities based solely on taxa abundance data. Finally, resulting groups were graphically presented on ordinations to interpret, a posteriori, the environmental gradients associated with each diatom group and to identify the diatom communities representing the reference conditions for each of the stream reach groups (Fig. 2). This procedure was performed for each ecoregion to discriminate, for example, between the reference diatom communities associated with circumneutral, acidic streams located on the Canadian Shield and communities representing the least-impacted conditions found in smaller, more alkaline agricultural streams of the St. Lawrence Lowlands.

All classifications were performed using a Kohonen SOM combined with Ward's hierarchic cluster analyses. A Kohonen SOM is one of the most well-known neural networks with unsupervised learning rules; it performs a topology-preserving projection of the data space onto a regular two-dimensional space. As for the ordination methods, SOMs reduce the number of dimensions with a minimum loss of information. The data are projected onto a rectangular grid map containing multiple hexagonal cells. Sites with similar characteristics are mapped in the same vicinity. The distance between each hexagonal cell is then represented on a U matrix (Euclidean or Bray–Curtis) (Ultsch 1993). The U matrix calculates the distances between neighbouring units and visually presents them as grey-scaled clusters on the SOMs (Kohonen 2001). A Ward linkage method (Legendre

Fig. 2. Schematic representation of the steps followed to determine the diatom-based reference conditions.

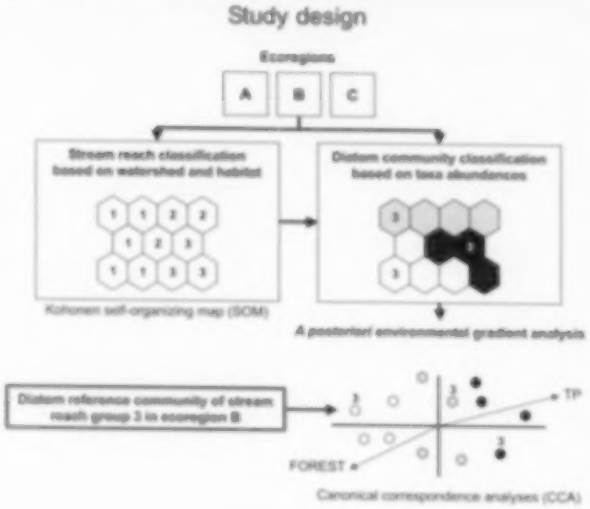

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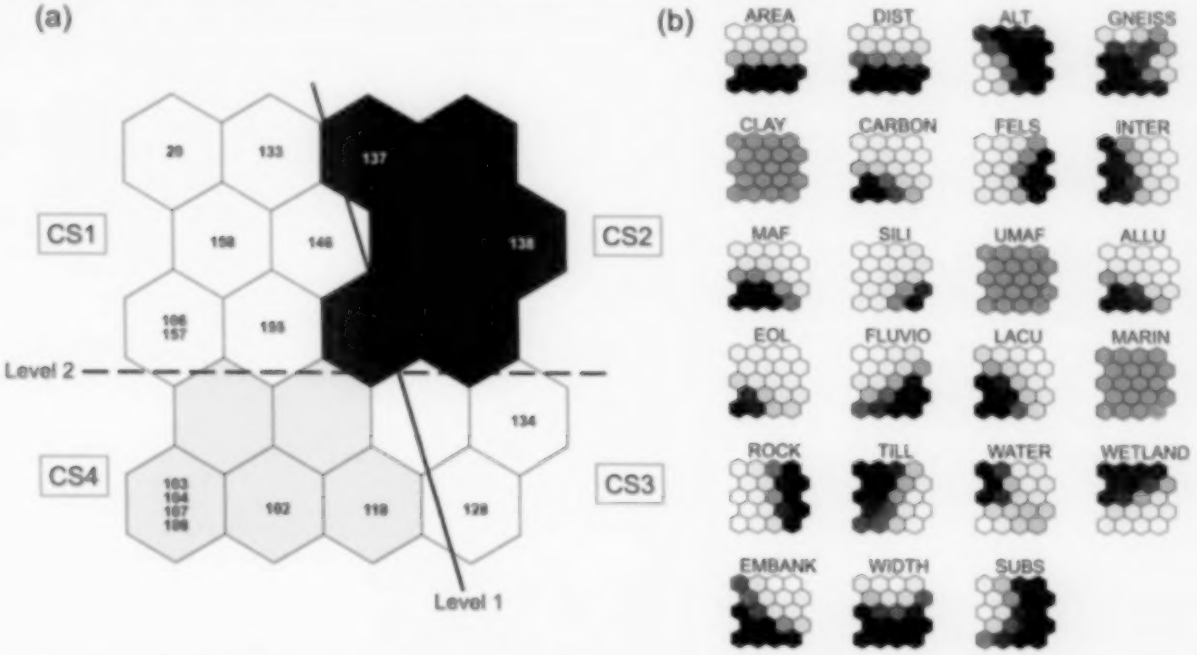
Fig. 3. (*a*) Kohonen self-organizing map (SOM) showing the four stream reach groups established for the Canadian Shield based on watershed and habitat characteristics and the two differentiation levels derived from the Ward's clustering method (the groups are described in Table 2). The numbers in the hexagonal cells represent the sampling site identification numbers (see Table 2). (*b*) SOM distribution map of environmental variables used to classify the stream reach groups. Dark cells represent high values, whereas light cells represent low values. The codes for the environmental variables are described in Appendix A.



and Legendre 1998), derived from the U matrix, was used to further classify the hexagonal cells in a reduced number of groups. A formula was used to calculate the number of cells needed to map the sites for each ecoregion (Y.-S. Park, personal communication, 2003). Kohonen SOMs were conducted using MATLAB software (MathWorks Inc. 2001) and the SOM toolbox developed by Sovan Lek's team at the Laboratoire Dynamique de la Biodiversité (Paul Sabatier University, Toulouse, France). Further information on SOM theory and its ecological applications can be found in Kohonen (2001) and Park et al. (2003). The SOM procedure has been used in a number of ecological studies and has been proven useful in the classification of aquatic communities (e.g., Giraudel and Lek 2001; Park et al. 2003, 2005) and the identification of reference conditions (Gosselain et al. 2005).

Diatom groups were graphically presented using canonical correspondence analyses (CCAs). The diatom community located at the lower extremity of the alteration gradient was considered to be the reference community. When several reference samples were available, the reference community was established by calculating the average relative abundance of each taxon. Preliminary CCAs were performed using all habitat- and watershed-related variables, excluding physico-chemical variables. Variables with a variance inflation factor (VIF) exceeding 10 were not included in the CCAs as they were highly correlated with other variables. Monte Carlo permutation tests were used to select variables explaining a significant portion of the variance ($p \leq 0.05$). A second series of CCAs was performed using only physico-chemical variables. The same procedure was repeated to eliminate multicollinearity and to select significant variables. A final CCA was conducted using selected physico-chemical variables and watershed-related variables. The selected watershed variables were included in the ordination as passive variables (added post hoc to the ordination by projection). All ordination analyses were performed using CANOCO 4.5 (ter Braak and Smilauer 2002).

Results

Stream reach classification for the Canadian Shield

The 17 sample sites from the Canadian Shield were classified into four groups sharing similar watershed and habitat characteristics (Fig. 3 and Table 2). Canadian Shield watersheds were found to be composed mainly of gneiss–paragneiss rocks covered by till. The stream widths were more than 5 m at all sites. The most important site separation (level 1 on the SOM) discriminated groups 1 and 4 from groups 2 and 3. The sites forming groups 1 and 4 had watersheds that consisted mainly of till and lacustrine deposits underlain by a higher proportion of intermediate rocks. The watersheds of groups 2 and 3 contained primarily fluvioglacial deposits and had a higher proportion of surficial bedrock (partly composed of felsic rocks). The sites forming groups 1 and 2 had smaller watersheds and narrower streambeds. Wetlands also occupied a significant portion of their watersheds. In the watersheds of group 4, alluvium and eolian deposits were underlain by a small proportion of carbonated rocks.

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Table 2. Stream reaches of the Canadian Shield: stream reach group description, diatom reference samples (in bold), and most abundant diatom taxa in reference communities (>2%).

River name	BQMA	Sampling sites	Latitude (°N)	Longitude (°W)	pH in reference conditions	Most abundant diatom taxa in reference community (mean abundance >2%)
CS1: Sites located at or near lake outlets						
Rivière des Envies (U)	5030113	20	46.84	–72.54	Circumneutral	1. *Achnanthidium minutissimum*
De la Petite Nation (U)	4040039	106	45.90	–75.09		2. *Tabellaria flocculosa*
Noire (U)	5040139	133	46.93	–72.12		3. *Brachysira microcephala*
L'Assomption (U)	5220017	146	46.29	–73.80		4. *Encyonopsis microcephala*
Maskinongé (U)	5260015	155	46.33	–73.36		5. *Fragilaria crotonensis*
Du Loup (U)	5280019	157	46.43	–72.97		6. *Brachysira procera*
Du Loup (M)	5280020	158	46.60	–73.19		7. *Fragilaria* sp. 1
CS2: Upstream reaches of the St. Charles River						
St. Charles (U)	5090003	137	46.86	–71.36	Circumneutral	1. *Achnanthidium minutissimum*
St. Charles (M)	5090016	138	46.91	–71.37		2. *Diatoma moniliformis*
						3. *Navicula notha*
						4. *Staurosira construens* var. *venter*
						5. *Encyonopsis microcephala*
						6. *Brachysira microcephala*
						7. *Achnanthes minutissima* var. *saprophila*
						8. *Tabellaria flocculosa*
						9. *Fragilaria capucina* form 5
						10. *Fragilaria capucina* form 6
						11. *Gomphonema sphaerophorum*
CS3: Upstream reaches of the St. Maurice River and Jacques-Cartier River						
St. Maurice (U)	5010386	128	47.56	–72.84	Circumneutral	1. *Achnanthidium minutissimum*
Jacques-Cartier (U)	5080004	134	47.07	–71.42		2. *Tabellaria flocculosa*
						3. *Brachysira microcephala*
						4. *Eunotia pectinalis*
						5. *Fragilaria capucina* form 5
						6. *Achnanthes minutissima* var. *saprophila*
						7. *Fragilaria capucina* form 3
CS4: Tributaries of the Ottawa River						
Du Nord (U)	4010010	102	45.91	–74.14	Circumneutral	1. *Achnanthidium minutissimum*
Rouge (D)	4020001	103	45.64	–74.69		2. *Tabellaria flocculosa*
Du Diable (U)	4020103	104	46.07	–74.63		3. *Brachysira microcephala*
Du Lièvre (U)	4060001	107	46.55	–75.50		4. *Fragilaria capucina* form 5
Du Lièvre (D)	4060004	108	45.59	–75.42		5. *Fragilaria capucina* form 6
Gatineau (U)	4080223	110	46.62	–75.92		6. *Fragilaria capucina* form 7

Note: BQMA, Banque de données sur la qualité du milieu aquatique. Positions: D, downstream; M, middle stream; U, upstream.

Stream reach classification for the Appalachians

The 29 sample sites located in the Appalachians were divided into four groups with similar watershed and habitat characteristics (Fig. 4 and Table 3). The surficial deposits of most sites located in Appalachian watersheds were dominated by till. The most significant site separation (level 1 on the SOM) discriminated groups 1 and 2 from groups 3 and 4. The sites belonging to groups 1 and 2 had larger watersheds (especially group 1) and wider stream beds and were underlain by a higher proportion of clay, mafic, and ultramafic rocks than those of groups 3 and 4. Lakes also occupied a large portion of the watersheds in groups 1 and 2 (e.g., Lake Memphremagog and Lake Brome). The watersheds of the sites located in the natural region of the Lower St. Lawrence Plain (groups 3 and 4; northeast of the Appalachians) contained mostly siliceous rocks. Sites upstream and downstream of the River Fouquette (group 4), located at the foot of the Appalachians, were distinct from all other groups because of their narrow stream beds (less than 2 m) and very small watershed size (50 km^2). Sites in this group had watersheds containing wetlands and consisted mainly of alluviums and marine deposits underlain by siliceous rocks.

Stream reach classification for the St. Lawrence Lowlands

The 80 sites sampled from the St. Lawrence Lowlands were classified into six groups with similar watershed and habitat characteristics (Fig. 5 and Table 4). Aside from

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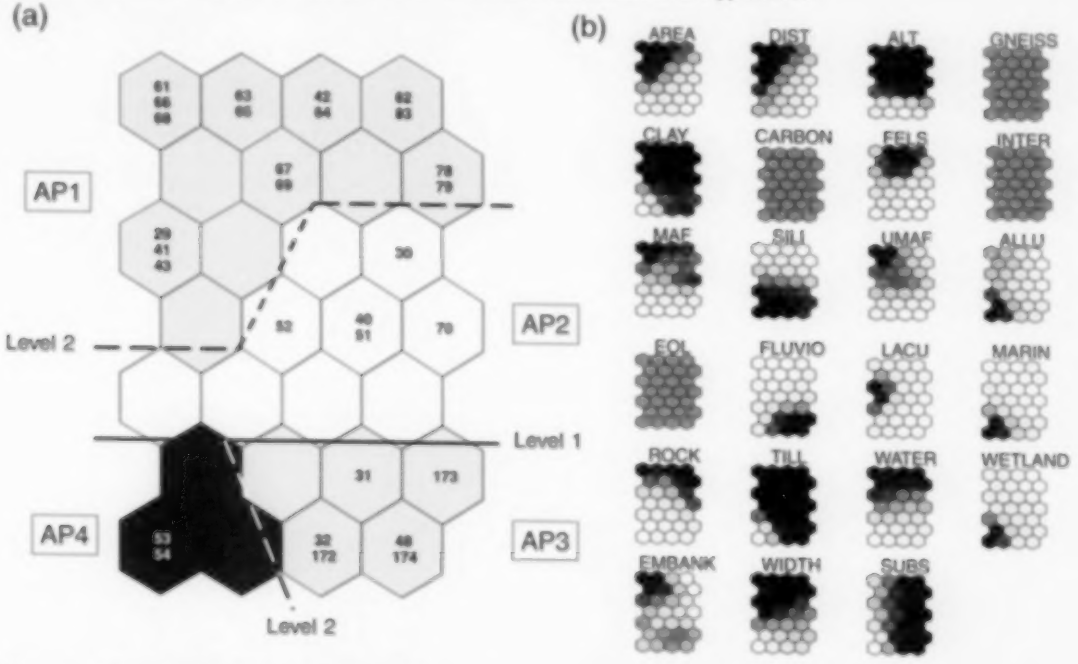
Fig. 4. (*a*) Kohonen self-organizing map (SOM) showing the four stream reach groups established for the Appalachians based on watershed and habitat characteristics and the two differentiation levels derived from the Ward's clustering method (the groups are described in Table 3). The numbers in the hexagonal cells represent the sampling site identification numbers (see Table 3). (*b*) SOM distribution map of environmental variables used to classify the stream reach groups. Dark cells represent high values, whereas light cells represent low values. The codes for the environmental variables are described in Appendix A.



group 2, most of the watersheds were dominated by marine deposits. The most important site separation (level 1 on the SOM) discriminated groups 1 and 2 from groups 3 to 6. Groups 1 and 2 represented wide rivers with large watersheds mostly located in an upstream ecoregion (Appalachians or Canadian Shield) containing a higher proportion of felsic rock. The watersheds of group 2 were dominated by gneiss–paragneiss rocks that are characteristic of the Canadian Shield. As a result, the water chemistry of some of the rivers that flow through the lowlands reflects the characteristics of an upstream ecoregion. The sites forming group 1 are all located on the Richelieu River. Groups 4 and 5 consisted of small streams of the St. Lawrence Plain watersheds covered mainly by marine deposits. The majority of the sites belonging to groups 3 and 4 were located in the natural region of the Upper St. Lawrence Plain, with watersheds consisting primarily of carbonated rocks. Sites belonging to group 5 were mainly located in the Middle St. Lawrence Plain and had watersheds consisting of siliceous and clay rocks covered by fluvioglacial and till deposits. These watersheds also had a higher proportion of wetlands. Watershed and stream sizes for groups 4 and 5 were generally smaller than those of groups 3 and 6.

Reference diatom communities of the Canadian Shield

The 29 diatom communities sampled on the Canadian Shield during the fall of 2002 and 2003 were classified into seven community types based solely on taxa relative abundances (Fig. 6). An a posteriori CCA was conducted using the seven groups to determine the direction of the environmental gradients influencing the structure of diatom communities on the Canadian Shield. Three outliers (altered communities) were removed from the ordination to improve the identification of reference communities. The physico-chemical variables that explained a statistically significant amount of the variation in diatom community structures were CON and CHL. The first four ordination axes summarized 26.7% of the variation observed in diatom communities and explained 61% of the relationship between taxa and selected environmental variables. The eigenvalue for the first axis (λ_1) was 0.31 and for the second axis (λ_2) was 0.24. Forested area and the proportion of alluviums were statistically significant variables characterizing watersheds and habitats and were included in the ordination as passive variables. Based on physico-chemistry, samples representing reference conditions were positioned on the left side of the ordination. The communities representing the most altered sites were located at the foot of the Canadian Shield where there was a substantial proportion of alluvium present (correlated with population and agriculture) in the watershed. Samples from diatom groups 5 and 6 were positioned on the left of the CCA ordination and therefore were used to define the reference communities for each of the Canadian Shield stream reach groups (the diatom reference sites are listed in Table 2, along with each community's most abundant taxa). When several reference samples were available, the reference community was established by calculating the average relative abundance of each taxon. For example, samples from Des Envies River (D20), De La Petite Nation River (B106 and D106), Noire River (B133), and Assomption

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Table 3. Stream reach groups of the Appalachians: stream reach group description, diatom reference samples (in bold), and most abundant diatom taxa in reference communities (>2%).

River name	BQMA	Sampling sites	Latitude (°N)	Longitude (°W)	pH in reference conditions	Most abundant diatom taxa in reference community (mean abundance > 2%)
AP1: Upstream reaches located in the Estrie–Beauce Complex						
Madawaska (U)	1170001	29	47.55	–68.64	Alkaline	1. *Achnanthidium minutissimum*
Chaudière (U)	2340004	41	46.18	–70.72		2. *Nitzschia sinuata* var. *tabellaria*
Chaudière (U)	2340006	42	45.69	–70.79		3. *Nitzschia fonticola*
Chaudière (M)	2340014	43	46.50	–71.07		4. *Nitzschia palea* var. *debilis*
St. François (U)	3020035	61	45.48	–71.94		5. *Encyonopsis microcephala*
St. François (U)	3020040	63	45.37	–71.85		6. *Fragilaria capucina* form 6
St. François (M)	3020081	66	45.66	–72.14		7. *A. minutissima* var. *saprophila*
Magog (U)	3020037	62	45.27	–72.10		8. *Fragilaria nanana*
Magog (M)	3020073	65	45.26	–72.16		9. *A.* cf. *latecephalum*
Magog (D)	3020176	68	45.40	–71.90		10. *Staurosira construens*
Au Saumon (U)	3020042	64	45.68	–71.40		11. *Fragilaria crotonensis*
Massawippi (D)	3020082	67	45.36	–71.86		
Coaticook (D)	3020177	69	45.31	–71.88		
Yamaska sud-est (U)	3030041	78	45.18	–72.66		
Yamaska (U)	3030094	79	45.28	–72.51		
Yamaska (U)	3030199	83	45.27	–72.80		
AP2: Upstream reaches with small watersheds located in the Estrie–Beauce Complex						
Cabano (D)	1170022	30	47.58	–68.92	Circumneutral	1. *Achnanthidium minutissimum*
Etchemin (U)	2330010	40	46.49	–70.45		2. *Fragilaria capucina* form 6
Bécancour (U)	2400005	51	46.05	–71.45		3. *Fragilaria capucina* form 5
Bécancour (U)	2400006	52	46.16	–71.56		4. *Encyonopsis microcephala*
Aux Cerises* (D)	3020187	70	45.29	–72.17		5. *Fragilaria capucina* form 7
						6. *A.* cf. *latecephalum*
						7. *Fragilaria capucina* form 4
						8. *Fragilaria capucina* form 3
AP3: Upstream reaches located in the Lower St. Lawrence Complex						
Du Loup (U)	2250002	31	47.58	–69.67	Circumneutral	1. *Achnanthidium minutissimum*
Du Loup (D)	2250005	32	47.84	–69.53		2. *Achnanthidium deflexum*
Dufour (U)	2260004	172	46.55	–71.88		3. *Fragilaria capucina* form 3
St. Denis (U)	2260005	173	46.54	–71.75		4. *Fragilaria capucina* form 6
Aux Perles (U)	2260006	174				3. *Staurosira construens* var. *venter*
Des Îles Brûlées (U)	2340086	48	46.51	–71.15		4. *Brachysira microcephala*
						5. *Fragilaria capucina* form 5
						6. *Fragilaria nanana*
						7. *Fragilaria capucina* form 7
						8. *Encyonopsis microcephala*
						9. *Cymbella delicatula*
AP4: Reaches of the Fouquette Stream						
Fouquette (D)	2E90001	53	47.71	–69.69	Alkaline	1. *Amphora pediculus*
Fouquette* (U)	2E90002	54	47.67	–69.66		2. *Navicula germainii*
						3. *Cocconeis placentula* var. *euglypta*
						4. *Navicula gregaria*
						5. *Achnanthidium minutissimum*
						6. *Rhoicosphenia abbreviata*
						7. *Meridion circulare*
						8. *Navicula cryptocephala*
						9. *Nitzschia sociabilis*
						10. *Navicula minima*

Note: BQMA, Banque de données sur la qualité du milieu aquatique. The asterisk (*) indicates that the reference community identified represents the least-disturbed conditions found and is not a true reference community. Positions: D, downstream; M, middle stream; U, upstream.

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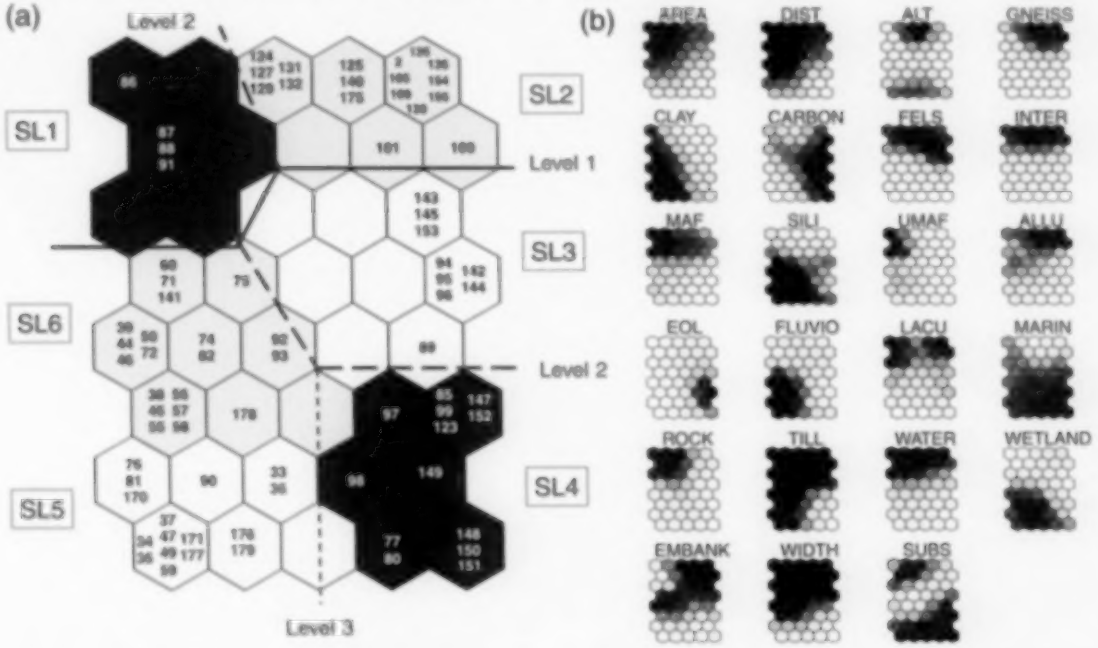
Fig. 5. (*a*) Kohonen self-organizing map (SOM) showing the six stream reach groups established for the St. Lawrence Lowlands based on watershed and habitat characteristics and the three differentiation levels derived from the Ward's clustering method (the groups are described in Table 4). The numbers in the hexagonal cells represent the sampling site identification numbers (see Table 4). (*b*) SOM distribution map of environmental variables used to classify the stream reach groups. Dark cells represent high values, whereas light cells represent low values. The codes for the environmental variables are described in Appendix A.



River (D146) all belong to stream reach group CS1 and were found on the left side of the CCA's first axis (Fig. 6). These samples represent the reference communities for other members of this group, such as the Maskinongé River (upstream) and Du Loup River (upstream). These rivers were located on the lower right portion of the ordination and are therefore more impacted than their reference sites. In the context of future restoration actions, one would expect that the diatom communities of these impacted rivers would change and resemble the reference community presented in Table 2.

Reference diatom communities of the Appalachians

The 50 diatom communities sampled in the Appalachians during the fall of 2002 and 2003 were classified into 10 type communities (Fig. 7). An a posteriori CCA was conducted using these 10 groups to determine the direction of the environmental gradients influencing the structure of diatom communities in the Appalachians. Four outliers (altered communities) were removed from the ordination to improve the identification of reference communities. The physico-chemical variables that explained a statistically significant amount of the variation in Appalachian diatom community structures were TP, CON, CHL, DOC, T, and O_2. The first four ordination axes summarized 16.6% of the variation observed in diatom communities and explained 79.3% of the relationship between taxa and the statistically significant environmental variables. The eigenvalues for the first and second axes were 0.31 and 0.2, respectively. Marine deposits, siliceous and ultramafic rocks, wetlands, and pastures were the watershed and habitat variables that explained a significant portion of the variation in diatom community structures and were included in the ordination as passive variables. The reference samples were positioned on the left portion of the ordination set, opposite to the pollution gradient. The communities representing the most altered sites were located at the foot of the Appalachians (marine deposits), where pasture areas are extensive. The samples from diatom groups 5 to 8 were positioned on the left portion of the CCA ordination and were used to define the reference communities for each stream reach group within the Appalachians (the diatom reference sites are listed in Table 3, along with each community's most abundant taxa).

Reference diatom communities of the St. Lawrence Lowlands

The 125 diatom communities sampled in the St. Lawrence Lowlands during the fall of 2002 and 2003 were classified into 10 type communities (Fig. 8). An a posteriori CCA was conducted using these 10 groups to determine the direction of the environmental gradients influencing the structure of diatom communities in the St. Lawrence Lowlands. Eleven outliers (altered communities) were removed from the ordination to improve the identification of reference communities. The physico-chemical variables that explained a statistically significant amount of the variation in diatom community were CON, pH, T, DOC, VEL, SS, and TUR. The first four ordination axes summarized 16.6% of the variation observed in diatom communities and explained 80.3% of the relationship between taxa and selected environmental variables. The eigenvalues for the first and second axes were

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Table 4. Stream reach groups of the St. Lawrence Lowlands: stream reach group description, diatom reference samples (in bold), and most abundant diatom taxa in reference communities (>2%).

River name	BQMA	Sampling sites	Latitude (°N)	Longitude (°W)	pH in reference conditions	Most abundant diatom taxa in reference community (mean abundance > 2%)
SL1: Reaches of the Richelieu River						
Richelieu (D)	3040009	86	46.02	–73.13	Alkaline	1. *Achnanthidium minutissimum*
Richelieu (D)	3040010	87	45.40	–73.25		2. *Cocconeis placentula* var. *euglypta*
Richelieu* (U)	3040012	88	45.06	–73.33		3. *Fragilaria capucina* var. *vaucheriae*
Richelieu (M)	3040017	91	45.69	–73.19		4. *Staurosirella pinnata*
						5. *Nitzschia fonticola*
						6. *Nitzschia palea* var. *debilis*
						7. *Pseudostaurosira binodis*
						8. *Pseudostaurosira brevistriata*
						9. *Cocconeis pediculus*
SL2: Downstream reaches of the St. Lawrence Lowlands with most of their watershed located in the Canadian Shield						
Rivière des Envies (D)	5030114	2	46.62	–72.41	Circumneutral	1. *Achnanthidium minutissimum*
Du Nord (M)	4010002	100	45.56	–74.34		2. *Fragilaria capucina* form 7
Du Nord (D)	4010008	101	45.72	–74.09		3. *A. minutissima* var. *saprophila*
De la Petite Nation (D)	4040001	105	45.61	–75.13		4. *Tabellaria flocculosa*
Gatineau (D)	4080003	109	45.49	–75.75		4. *Fragilaria capucina* form 3
St. Maurice (D)	5010007	124	46.38	–72.61		5. *Navicula notha*
Shawinigan (D)	5010012	125	46.54	–72.77		6. *Fragilaria capucina* form 5
St. Maurice (M)	5010014	127	46.54	–72.75		7. *Brachysira microcephala*
Batiscan (D)	5030001	129	46.53	–72.34		8. *Fragilaria capucina* form 6
St. Anne (D)	5040007	130	46.57	–72.21		9. *Gomphonema manubrium*
St. Anne (U)	5040113	131	46.90	–71.85		10. *Nitzschia palea* var. *debilis*
St. Anne (M)	5040116	132	46.82	–71.97		11. *Adlafia* cf. *bryophila*
Jacques-Cartier (D)	5080006	135	46.68	–71.75		
St. Charles (D)	5090002	136	46.81	–71.26		
L'Assomption (D)	5220001	140	46.04	–73.44		
Maskinongé (D)	5260003	154	46.18	–73.03		
Du Loup (D)	5280001	156	46.24	–72.92		
Blanche (D)	5040006	175	46.03	–72.88		
SL3: Reaches of the Upper St. Lawrence Lowlands with most of their watershed overlying carbonated rocks						
L'Acadie (D)	3040013	89	45.43	–73.35	Alkaline	1. *Nitzschia sinuata* var. *tabellaria*
Chateauguay (M)	3090003	94	45.11	–74.09		2. *Achnanthidium minutissimum*
Chateauguay (U)	3090005	95	45.02	–74.17		3. *A.* cf. *latecephalum*
Trout River (U)	3090009	96	45.01	–74.30		4. *Navicula capitatoradiata*
L'Assomption (M)	5220004	142	45.94	–73.40		5. *Cymbella excisa* var. *procera*
De l'Achigan (D)	5220005	143	45.85	–73.45		6. *Gomphonema entolejum*
St. Esprit (D)	5220006	144	45.86	–73.46		7. *A. minutissima* var. *saprophila*
Ouareau (D)	5220012	145	45.95	–73.41		8. *Melosira varians*
Bayonne (D)	5240001	153	46.09	–73.17		9. *Geissleria decussis*
						10. *Staurosira construens* var. *venter*
SL4: Reaches of small streams of the Upper St. Lawrence Lowlands with part of their watershed overlying carbonated rocks						
Des Hurons (M)	3040007	85	45.49	–73.19	Alkaline	1. *Achnanthidium minutissimum*
Ruisseau Norton (M)	3090046	97	45.16	–73.68		2. *A. minutissima* var. *saprophila*
Des Anglais (U)	3090047	98	45.00	–73.65		3. *Nitzschia fonticola*
Ruisseau St. Louis (D)	3110003	99	45.27	–73.90		4. *Fragilaria capucina* var. *vaucheriae*
Mascouche (D)	4640003	123	45.72	–73.58		5. *Nitzschia palea* var. *debilis*
Ruisseau du Point-du-Jour (D)	5220063	147	45.85	–73.41		6. *Navicula minima*
Ruisseau Vacher (M)	5220239	148	45.93	–73.51		7. *Navicula sp. 10*
Ruisseau St. Pierre (M)	5220240	149	45.98	–73.44		
Ruisseau St. Esprit (M)	5220241	150	45.93	–73.62		

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Table 4 (*concluded*).

River name	BQMA	Sampling sites	Latitude (°N)	Longitude (°W)	pH in reference conditions	Most abundant diatom taxa in reference community (mean abundance > 2%)
SL5: Reaches of small streams with most of their watershed located in the middle St. Lawrence Lowlands						
Boyer Sud (D)	2300002	34	46.72	–70.98	Alkaline	1. *Achnanthidium minutissimum*
Boyer Nord (D)	2300003	35	46.70	–71.00		2. *Cocconeis placentula* var. *euglypta*
Ruisseau du Portage (D)	2300004	36	46.79	–70.91		3. *Staurosirella pinnata*
Ruisseau Honfleur (D)	2300005	37	46.69	–70.93		4. *Nitzschia palea* var. *debilis*
Bras d'Henri (U)	2340051	47	46.54	–71.34		5. *Navicula capitatoradiata*
Bras d'Henri (D)	2340099	49	46.51	–71.22		6. *Nitzschia fonticola*
Des Pins (D)	3010038	59	46.00	–72.03		7. *Navicula germainii*
Yamaska Sud-Est* (D)	3030031	76	45.27	–72.92		8. *A. minutissima* var. *saprophila*
Yamaska Nord (D)	3030108	81	45.33	–72.81		9. *Encyonema silesiacum*
Aux Brochets (D)	3040015	90	45.12	–73.07		10. *Navicula cryptocephala*
La Chaloupe (D)	5230001	152	46.07	–73.18		11. *Planothidium lanceolatum*
Aux Perles (D)	2260002	170	46.29	–72.18		12. *Cyclotella meneghiniana*
Goudron (D)	2260003	171	46.18	–71.95		13. *Fragilaria capucina* var. *vaucheriae*
Gentilly* (M)	QC1	176	46.28	–72.18		
Rosaire (D)	QC2	177	46.18	–71.95		
Du Bois Clair* (U)	QC4	179	46.54	–71.75		
SL6: Reaches of large rivers located in the St. Lawrence Lowlands with most of their watershed in the Appalachians						
Chaudière* (D)	2340033	44	46.70	–71.28	Alkaline	1. *Achnanthidium minutissimum*
Beaurivage (D)	2340034	45	46.65	–71.30		2. *Nitzschia palea* var. *debilis*
Bécancour (D)	2400004	50	46.35	–72.44		3. *Nitzschia fonticola*
Nicolet (U)	3010007	55	46.00	–72.09		4. *Cocconeis pediculus*
Nicolet* (D)	3010008	56	46.15	–72.54		5. *A. minutissima* var. *saprophila*
Nicolet Sud-Ouest (D)	3010009	57	46.13	–72.60		6. *Cocconeis placentula* var. *euglypta*
Nicolet Sud-Ouest (U)	3010036	58	45.88	–72.23		7. *Nitzschia sinuata* var. *tabellaria*
St-François (D)	3020031	60	46.07	–72.82		8. *Navicula capitatoradiata*
St-François (D)	3020243	71	45.93	–72.50		9. *Nitzschia palea* var. *debilis*
Noire (M)	3030003	72	45.50	–72.90		
Yamaska (D)	3030023	74	46.00	–72.91		
Yamaska (D)	3030026	75	45.52	–72.98		
Yamaska (M)	3030123	82	45.78	–72.88		
Chateauguay (D)	3090001	92	45.29	–73.80		
Des Anglais (D)	3090002	93	45.18	–73.85		
L'Assomption (D)	5220003	141	45.75	–73.47		
Du Chêne (D)	QC3	178	46.55	–71.87		

Note: BQMA, Banque de données sur la qualité du milieu aquatique. The asterisk (*) indicates that the reference community identified represents the least-disturbed conditions found and is not a true reference community. Positions: D, downstream; M, middle stream; U, upstream.

0.38 and 0.22, respectively. Population, forested area, marine deposits, carbonated rocks, gneiss–paragneiss rocks, felsic rocks, and intermediate rocks were the watershed and habitat variables that explained a significant amount of the variation in diatom community structures and were included in the ordination as passive variables. Based on physico-chemistry, samples representing reference conditions were positioned on the right portion of the ordination. The samples from diatom groups 3 to 8 were positioned on the right portion of the CCA ordination and were used to define the reference communities for each stream reach group within the St. Lawrence Lowlands (the diatom reference sites and each community's most abundant taxa are listed in Table 4).

Combined analysis of all diatom reference communities

From the examination of Tables 2 to 4, we observed that reference diatom communities may exhibit strong similarities from one stream reach group to another. For example, all reference communities of the stream reach groups from the Canadian Shield are similarly dominated by *Achnanthidium minutissimum* and *Tabellaria flocculosa*. Moreover, reference communities may be similar from one ecoregion to another. For example, reference communities from the Appalachian stream groups AP2 and AP3 have a taxonomic composition similar to the reference communities of the stream group SL2 in the St. Lawrence Lowlands (*A. minutissimum* and different forms of *Fragilaria capucina*). To test the similarities between reference communities, we conducted a combined analysis of all reference diatom communities from the stream reach groups of the three ecoregions. The SOM classified all of the 14 diatom reference communities into only four communities (Fig. 9). An a posteriori CCA was conducted using all reference diatom communities to determine the direction of the environmental gradients influencing the structure of diatom reference communities (Fig. 10). The physico-chemical variables explaining a significant amount of the variation in diatom community structures were pH and CON. CON is correlated with FC, TN,

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Fig. 6. (*a*) Kohonen self-organizing map (SOM) showing the seven diatom communities established for the Canadian Shield based solely on taxa relative abundances and the three differentiation levels derived from the Ward's clustering method. Median values for water chemistry are presented in boxes for each community. The numbers in the hexagonal cells represent the sampling site identification numbers (see Table 2). Sites that were sampled in 2002 are coded as "B" and those sampled in 2003 are coded as "D". (*b*) Canonical correspondence analysis (CCA) sites scores. The diatom SOM groups are represented by symbols. The numbers in parentheses indicate the corresponding stream reach groups (see Fig. 3). The codes for the environmental variables are described in Appendix A (broken arrows indicate passive variables).

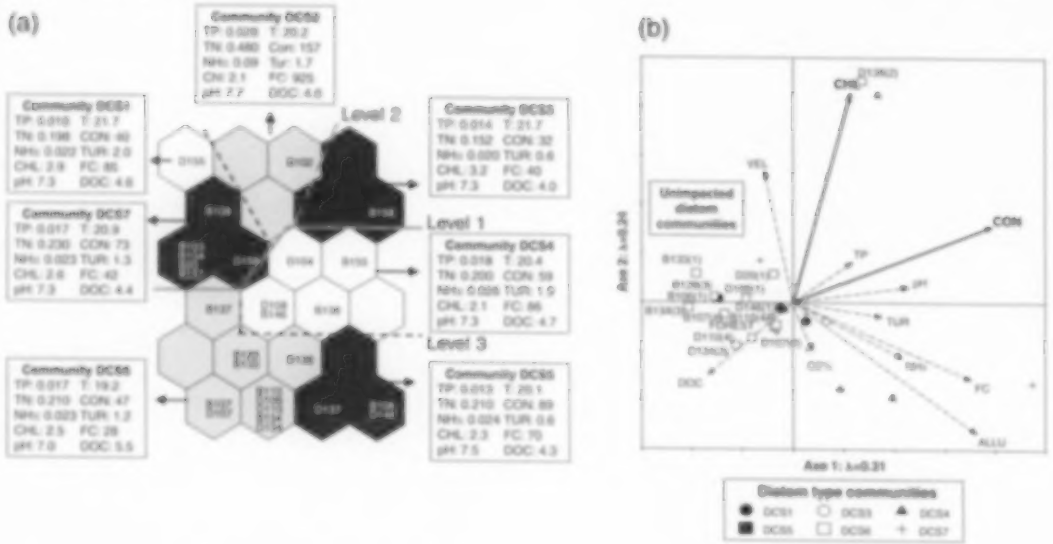

Fig. 7. (*a*) Kohonen self-organizing map (SOM) showing the 10 diatom communities established for the Appalachians based solely on taxa relative abundances and the four differentiation levels derived from the Ward's clustering. Median values for water chemistry are presented in boxes for each community. The numbers in the hexagonal cells represent the sampling site identification numbers (see Table 3). Sites that were sampled in 2002 are coded as "B" and those sampled in 2003 are coded as "D". (*b*) Canonical correspondence analysis (CCA) sites scores. The diatom SOM groups are represented by symbols. The numbers in parentheses indicate the corresponding stream reach groups (see Fig. 4). The codes for the environmental variables are described in Appendix A (broken arrows indicate passive variables).

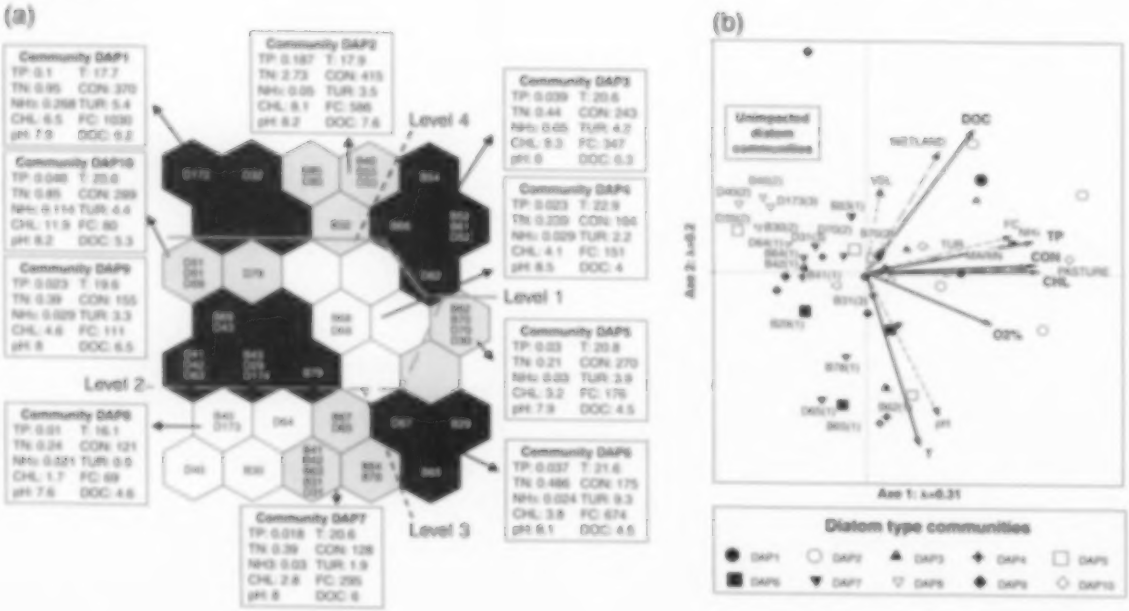

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Fig. 8. (*a*) Kohonen self-organizing map (SOM) showing the 10 diatom communities established for the St. Lawrence Lowlands based solely on taxa relative abundances and the four differentiation levels derived from the Ward's clustering method. Median values for water chemistry are presented in boxes for each community. The numbers in the hexagonal cells represent the sampling site identification numbers (see Table 4). Sites that were sampled in 2002 are coded as "B" and those sampled in 2003 are coded as "D". (*b*) Canonical correspondence analysis (CCA) sites scores. The diatom SOM groups are represented by symbols. The numbers in parentheses indicate the corresponding stream reach groups (see Fig. 5). The codes for the environmental variables are described in Appendix A (broken arrows indicate passive variables).

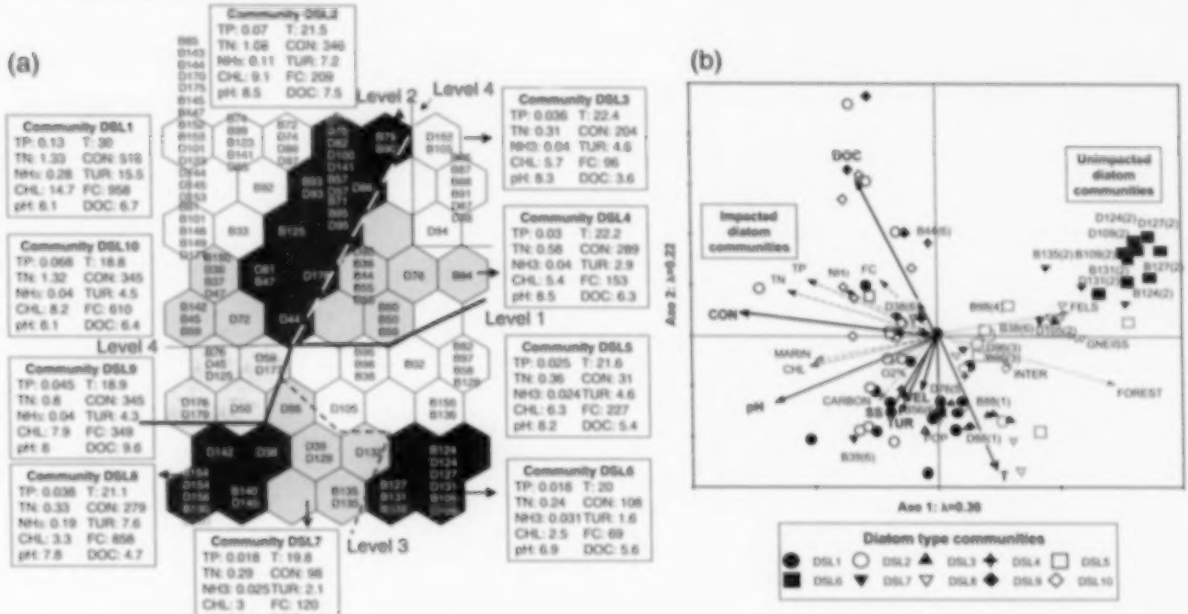

Fig. 9. Kohonen self-organizing map (SOM) showing the combined analysis of all diatom reference communities in the three ecoregions. The SOM classified all the diatom reference communities into four communities. The two differentiation levels derived from the Ward's clustering method are indicated. Median values for water chemistry are presented in boxes for each community. The underlined numbers represent the Canadian Shield sampling sites (Table 2), the numbers in italic represent the Appalachians sampling sites (Table 3) and the regular font represents the St. Lawrence sampling sites (Table 4). Sites that were sampled in 2002 are coded as "B" and those sampled in 2003 are coded as "D".

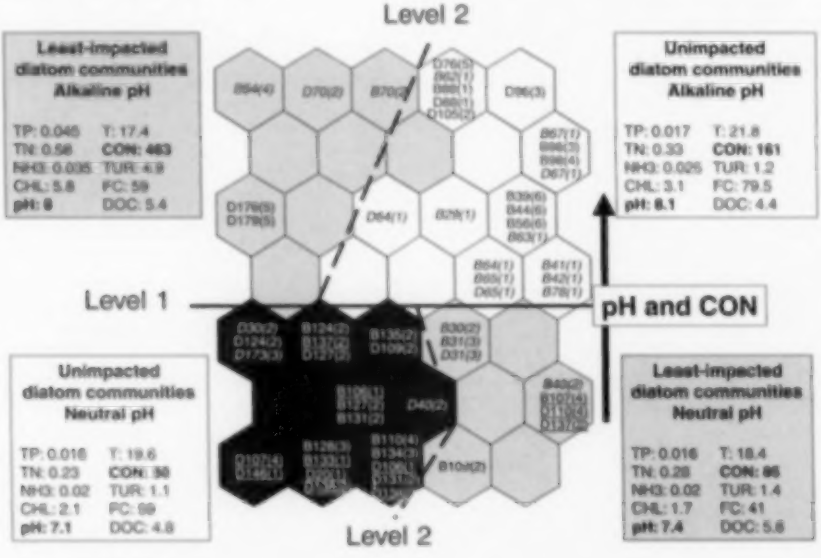

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Fig. 6. (*a*) Kohonen self-organizing map (SOM) showing the seven diatom communities established for the Canadian Shield based solely on taxa relative abundances and the three differentiation levels derived from the Ward's clustering method. Median values for water chemistry are presented in boxes for each community. The numbers in the hexagonal cells represent the sampling site identification numbers (see Table 2). Sites that were sampled in 2002 are coded as "B" and those sampled in 2003 are coded as "D". (*b*) Canonical correspondence analysis (CCA) sites scores. The diatom SOM groups are represented by symbols. The numbers in parentheses indicate the corresponding stream reach groups (see Fig. 3). The codes for the environmental variables are described in Appendix A (broken arrows indicate passive variables).

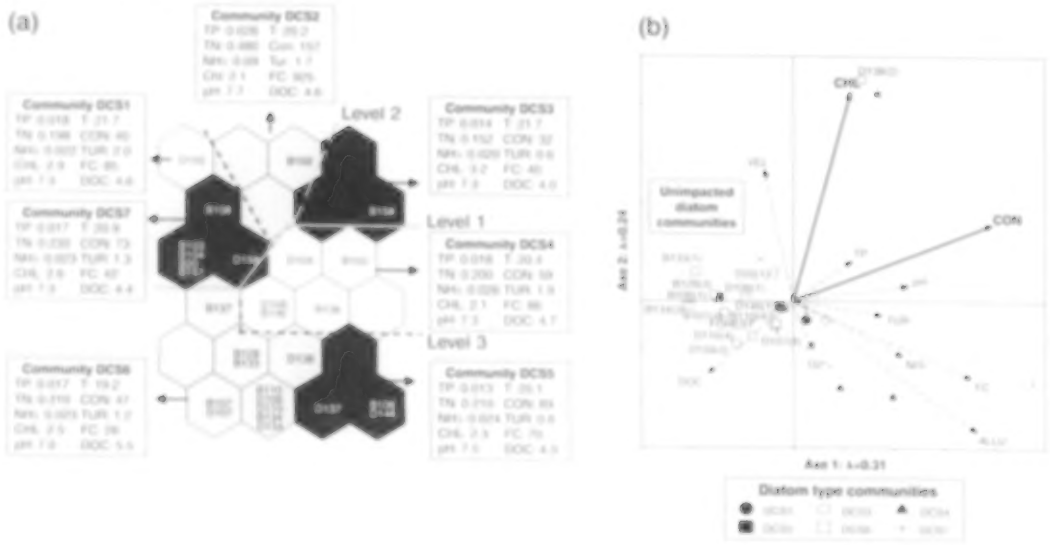

Fig. 7. (*a*) Kohonen self-organizing map (SOM) showing the 10 diatom communities established for the Appalachians based solely on taxa relative abundances and the four differentiation levels derived from the Ward's clustering. Median values for water chemistry are presented in boxes for each community. The numbers in the hexagonal cells represent the sampling site identification numbers (see Table 3). Sites that were sampled in 2002 are coded as "B" and those sampled in 2003 are coded as "D". (*b*) Canonical correspondence analysis (CCA) sites scores. The diatom SOM groups are represented by symbols. The numbers in parentheses indicate the corresponding stream reach groups (see Fig. 4). The codes for the environmental variables are described in Appendix A (broken arrows indicate passive variables).

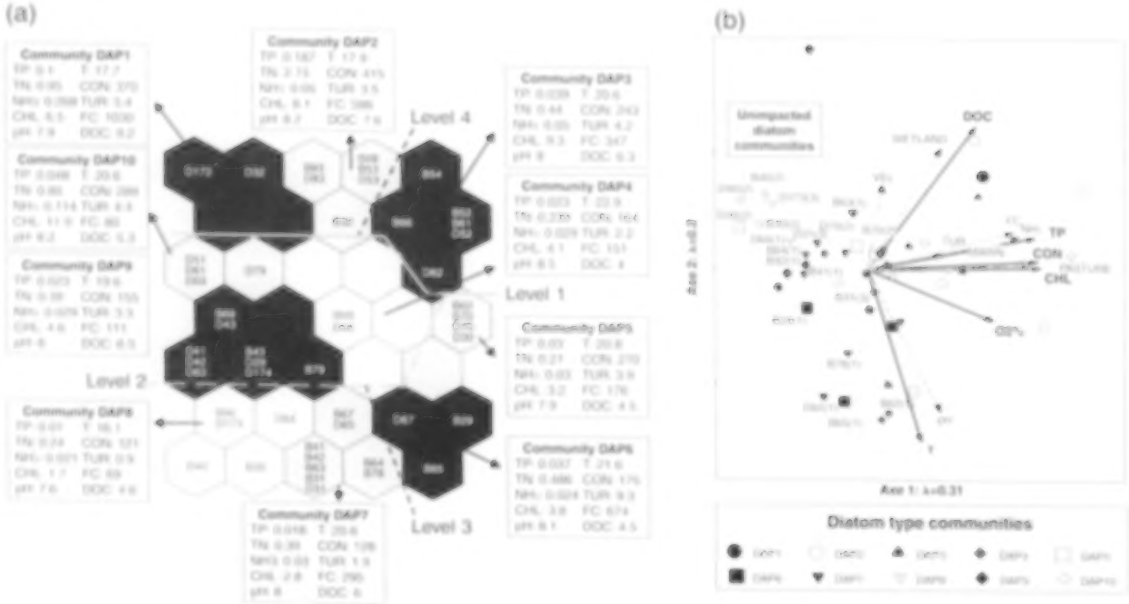

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Fig. 8. (*a*) Kohonen self-organizing map (SOM) showing the 10 diatom communities established for the St. Lawrence Lowlands based solely on taxa relative abundances and the four differentiation levels derived from the Ward's clustering method. Median values for water chemistry are presented in boxes for each community. The numbers in the hexagonal cells represent the sampling site identification numbers (see Table 4). Sites that were sampled in 2002 are coded as "B" and those sampled in 2003 are coded as "D". (*b*) Canonical correspondence analysis (CCA) sites scores. The diatom SOM groups are represented by symbols. The numbers in parentheses indicate the corresponding stream reach groups (see Fig. 5). The codes for the environmental variables are described in Appendix A (broken arrows indicate passive variables).

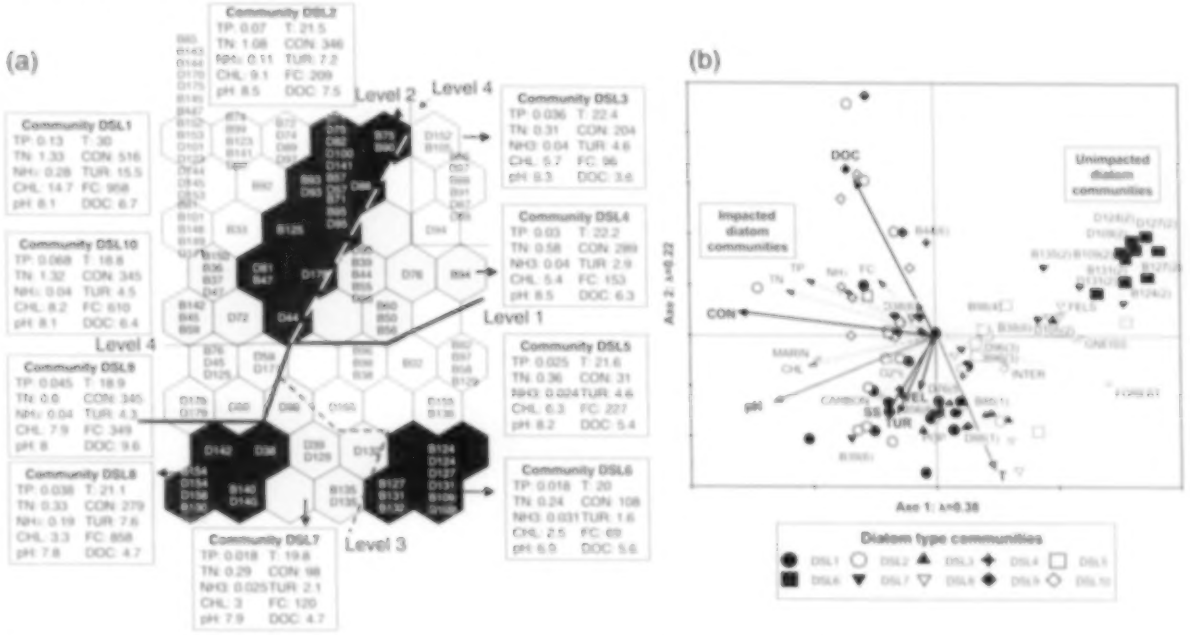

Fig. 9. Kohonen self-organizing map (SOM) showing the combined analysis of all diatom reference communities in the three ecoregions. The SOM classified all the diatom reference communities into four communities. The two differentiation levels derived from the Ward's clustering method are indicated. Median values for water chemistry are presented in boxes for each community. The underlined numbers represent the Canadian Shield sampling sites (Table 2), the numbers in italic represent the Appalachians sampling sites (Table 3) and the regular font represents the St. Lawrence sampling sites (Table 4). Sites that were sampled in 2002 are coded as "B" and those sampled in 2003 are coded as "D".

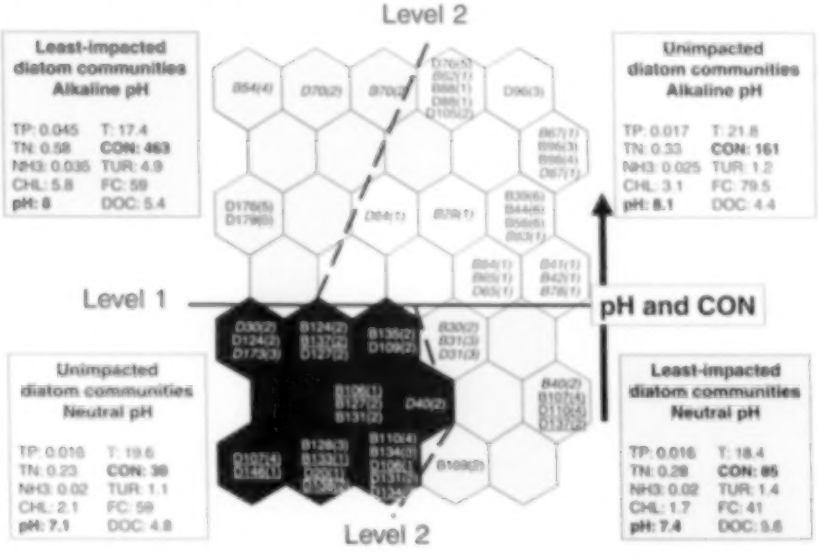

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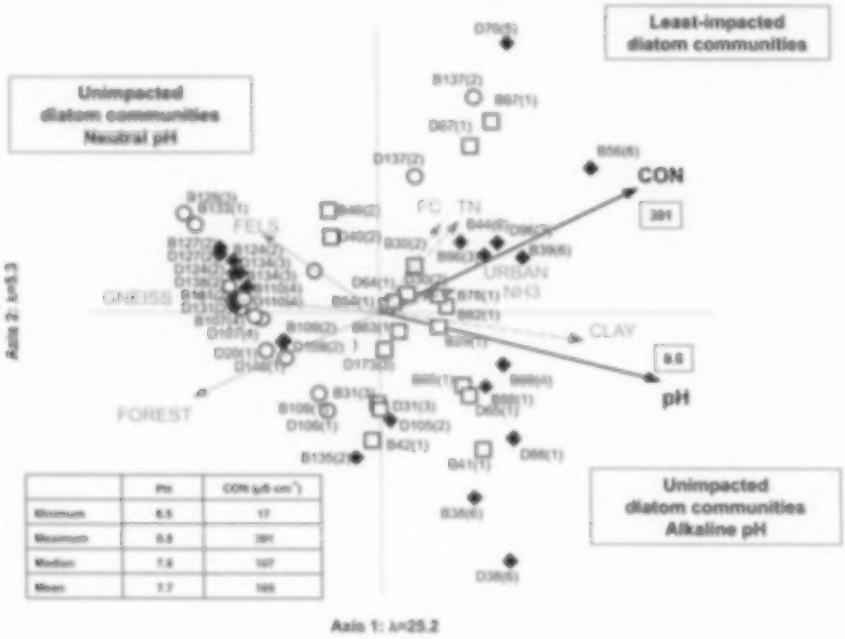
Fig. 10. Canonical correspondence analysis (CCA) sites scores showing the combined analysis of all diatom reference communities in the three ecoregions. The numbers represent the sampling site identification numbers (Tables 2–4). Sites that were sampled in 2002 are coded as "B" and those sampled in 2003 are coded as "D". The numbers in parentheses indicate the corresponding stream reach groups (Tables 2–4). The symbols represent the three ecoregions (circles, Canadian Shield; diamonds, St. Lawrence Lowlands; squares, Appalachians). The codes for the environmental variables are described in Appendix A (broken arrows indicate passive variables).



and NH_3, which indicates that conductivity is related to a degradation gradient. These correlated variables were included in the ordination as passive variables. The first four ordination axes summarized 22.6% of the variation observed in diatom communities. The eigenvalue for the first axis (λ_1) was 0.26 and for the second axis (λ_2) was 0.05. Forested area, urban area, and the presence of gneiss–paragneiss and felsic rocks were the significant variables characterizing watersheds and habitats and were included in the ordination as passive variables. The presence of gneiss–paragneiss and felsic rocks is inversely correlated with the presence of clay rocks. This relationship may indicate that natural alkaline pH could be explained by the high proportion of clay rocks in these watersheds. The most important site separation (level 1 on the SOM and axis 1 on the CCA) discriminated reference communities based on pH and conductivity. Samples on the left side of the ordination represent reference communities for the streams with naturally neutral pH and lower conductivity, whereas samples on the right side of the ordination represent reference communities for the streams with naturally alkaline pH and higher conductivity. A pH of 7.65 represents the separation line between both reference communities. Samples on the upper part of the ordination were slightly altered or had a higher conductivity in their natural state, but represent the least-impacted conditions for some stream reach groups of the Appalachians and the St. Lawrence Lowlands. Although a specific diatom reference community may be identified for each stream reach group, these results suggest that many of these communities exhibit strong similarities. As a result, only two reference communities may be used, one for circumneutral conditions and the other for alkaline conditions.

Discussion

Establishing reference conditions: abiotic and biotic approaches

In the reference-condition approach, a test site is compared with an appropriate set of reference sites characterizing the biological condition of a region. Two major analytical approaches for the comparison of test sites with reference conditions have been used so far: abiotic and biotic methods. Abiotic methods classify reference sites based on geographic and physical attributes. Some authors consider watershed variables as being the most useful spatial framework for aquatic ecosystem management (e.g., US Water Environment Federation 1992; Maxwell et al. 1995). However, because climate, geology, soil, and vegetation type are variables that are not specific to a single watershed, most authors consider ecoregions to constitute a superior spatial framework for the determination of water quality standards and restoration goals. Reference sites are chosen from streams with catchments belonging to specific ecoregions or subecoregions (e.g., Barbour et al. 1995, 1996). These regions are predefined using geomorphological characteristics such as climate, physiography, geology, soils, and vegetation (Omernik 1987). Some authors consider the combination of both the ecoregion and the watershed as necessary for the development of a regional reference site network of watersheds with similar reference communities within the same

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ecoregion (e.g., Hughes 1995; Omernik and Bailey 1997; Rogers and Wasson 1997). The ecoregion scheme has been widely used in multimetric methods to study macroinvertebrate communities. Several studies concluded that significant biotic variation among sites was related to ecoregion, especially where there were marked differences in topography between ecoregions (e.g., Gerritsen et al. 2000). However, although significant, the amount of variation related to landscape features is usually quite low (Hawkins et al. 2000). Local habitat features appear to account for more biotic variation than larger-scale environmental features. In the case of diatom communities, the study of Pan et al. (2000) showed that diatom community structure in reference streams did not vary with either ecoregion or catchment. As pointed out by Hawkins et al. (2000), classification based on both stream reach-level and larger-scale landscape features may provide a better tool for the prediction of aquatic community composition. In France, for example, 22 hydro-ecoregions were visually separated according to natural discontinuities in stream typology (Wasson et al. 2002). The final typology was, however, realized by evaluating biological reference conditions. Their results showed a good correspondence between the 22 hydro-ecoregions, the invertebrate communities, and the diatom communities. Different European countries are currently dividing their territory into "subecoregions" or aquatic "landscape units" (e.g., Austria: Fink et al. 2000). According to Hering et al. (2003), these classifications led to the establishment of about 100 stream types in Europe. Although the method and the variables used may differ from the above studies, our work shares the common approach of determining stream typology before the identification of the reference conditions specific to each typology.

Contrary to abiotic methods, biotic approaches make no a priori assumptions about the similarity of biological communities at different sites. Rather, the reference sites are classified using clustering methods based on the similarity of their species composition (Reynoldson et al. 1997). A method is then required to match a test site to the appropriate reference group. These reference sites can then be used to predict the community structure expected at the test site following restoration. The predictive model may be based, for example, on a discriminant function. Such biotic approaches were used for the development of bioassessment tools such RIVPACS (Wright et al. 1993), AusRivAS (Parsons and Norris 1996), and BEAST (Reynoldson et al. 1995).

Recent studies carried out in Europe (Coste et al. 2004; Descy et al. 2005; Gosselain et al. 2005) also investigated the identification of stream reference conditions based on biota. Reference sites for the French territory were first selected according to the floristic composition of diatom communities. Only the sites that did not experience environmental pressures were considered. Diatom-based index values were then calculated using the specific polluosensitivity index (IPS: Indice de Polluo-sensibilité Spécifique; Coste 1982) and the biological diatom index (IBD: Indice Biologique Diatomées; Lenoir and Coste 1996) for each of the selected reference sites. The index values for the selected reference sites were considered to be in a "good ecological state". In our opinion, the procedure of reference site selection based on diatom indices (IPS and IBD) is biased because of the development of indices that do not consider ecoregions and stream reach group variables. Reference communities for a specific type of environment may, therefore, not be adequately represented in the diatom index. Furthermore, the IPS and IBD were developed on the basis of the relationships between diatom communities and physico-chemistry data, which imply that the definition of the reference communities is dependent on the physico-chemical conditions of the stream. This circular argument disregards the use of nonredundant information in the characterisation of diatom community structure when attempting to supply additional information on the ecosystem's status. Finally, the identification of environmental pressures on each ecoregion was based on land use analysis. Although this method is certainly valid, there is a possibility that certain sources of degradation may not be detected and that the gradient discriminating between reference, intermediate, and degraded conditions may not be adequately identified.

The method used in our study combines abiotic and biotic classifications. The two-step procedure presented in this study was first used to classify stream reaches as a function of their natural watershed and habitat characteristics, both of which are known to influence diatom communities. This classification was conducted for each of the three ecoregions and identified four stream reach groups on the Canadian Shield, four in the Appalachians, and six in the St. Lawrence Lowlands. In parallel, diatom communities were classified based solely on taxa abundance data. This classification was also conducted for each ecoregion and identified seven diatom communities typical of the Canadian Shield, 10 diatom communities typical of the Appalachians, and 10 diatom communities typical of the St. Lawrence Lowlands. Resulting groups were graphically presented on ordinations to interpret, a posteriori, the environmental gradients associated with the diatom groups and to identify the diatom communities representing the reference conditions of each of the stream reach groups. The reference community for each stream reach group was found at the lower end of the alteration gradient, indicating nonimpacted or "least-disturbed" conditions. As a result, the selection of reference samples was based solely on community structure. Water physico-chemistry and land use characteristics were used only to interpret the position of each diatom community along the pollution gradient.

Finally, a classification based solely on diatom reference communities found pH and conductivity to be the main discriminating factors, regardless of ecoregion and stream type. Although a specific diatom reference community may be identified for each stream group, our results suggest that many of these communities exhibit strong similarities. Therefore, only two reference communities may be used, one for circumneutral conditions and one for alkaline conditions. These results suggest that pH and conductivity, which partially depend on geology and the presence of wetlands, have a major influence on the composition of reference diatom communities. Similar results were obtained in Europe and the USA (e.g., Potapova and Charles 2002; Gosselain et al. 2005).

Although only two reference communities are sufficient, it is still useful to compare a test site with its specified stream reach group reference community, especially where there is a lack of pristine conditions. For example, in the case of al-

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kaline conditions, most of the reference sites are from large rivers. These reference communities may not adequately represent the expected diatom community in small, unaltered streams. It is therefore useful to go back to the reference community identified for the stream reach group representing small agricultural streams. This reference community represents least-disturbed conditions rather than pristine conditions. It defines an intermediate restoration goal, which may be more realistic than the pristine conditions, especially for the heavily impacted streams of the St. Lawrence Lowlands.

Low pH reference diatom communities (pH below 7.65)

In Quebec, the presence of gneiss–paragneiss rocks and felsic rocks, with low buffering capacity, seems to be responsible for pH variations in the reference streams. This applied primarily to Canadian Shield reference sites and St. Lawrence Lowlands reference sites, which have a large portion of their watershed located on the Canadian Shield. Low pH also seems to be partly explained by the presence of ultramafic and siliceous rocks, although these relationships need to be confirmed by other analyses based solely on Appalachian reference sites. Finally, the presence of wetlands appears to explain the high concentrations of DOC in some watersheds, which contribute to lower the pH level, especially in the Appalachians.

As a result, reference communities identified for the streams with low buffering capacity in the Appalachians and St. Lawrence Lowlands have similar communities to those found on the Canadian Shield. Low pH reference diatom communities are dominated by taxa indicators of oligotrophic conditions, e.g., *A. minutissimum* (Leland 1994) and *F. capucina*, and more acidic conditions, e.g., *T. flocculosa* (Van Dam et al. 1994) and *Brachysira microcephala* (Reavie and Smol 2001). According to their taxonomic composition, almost all reference communities identified in Canadian Shield and Appalachian watersheds approach undisturbed biotypes.

High pH reference diatom communities (pH above 7.65)

Conversely, alkaline streams of the Appalachians have reference communities similar to those found in alkaline streams of the St. Lawrence Lowlands. Their high pH seems to be explained by the presence of carbonated and (or) clay rocks. These reference communities are dominated by such species as *A. minutissimum*, *Nitzschia sinuata* var. *tabellaria*, *Nitzschia fonticola*, and *Nitzschia palea* var. *debilis*. However, in the Appalachians, there is an absence of undisturbed reference samples for group 4 represented by the sites of the River Fouquette. The similarities between watershed characteristics of the River Fouquette and those of the small rivers located in the middle St. Lawrence Lowlands (group 5) suggests that they share the same restoration goals. However, wetlands occupy a significant portion of the River Fouquette's watershed (4.8%), potentially contributing to the acidification of the water and suggesting that the reference conditions may instead be acidic. More reference sites are needed to clarify this assumption. Moreover, the insufficient number or the lack of reference samples for certain stream categories, particularly in the St. Lawrence Lowlands (small agricultural streams), made it difficult to identify real restoration goals for these environments. Most of the taxa found in reference communities of small agricultural streams are also indicators of impacted conditions, e.g., *Cocconeis placentula* var. *euglypta* and *Planothidium lanceolatum*. An increase in the number of reference sites is necessary to clarify the structure of these reference communities.

Development of a bioassessment tool

In Canada, benthic macroinvertebrates are the group of aquatic organisms most widely used in bioassessment (e.g., Linke et al. 1999; Reynoldson et al. 2001; Winter et al. 2002). Several recent studies carried out in Canada and United States show the potential of diatom communities as indicators of water quality (e.g., Leland and Porter 2000; Winter and Duthie 2000*b*; Potapova and Charles 2002). Despite the fact that diatoms have been proven to be good indicators of environmental conditions, there are no diatom indices currently being used in Canadian biomonitoring programs. European countries have a long history of developing diatom-based indices for biological assessment and biocriteria. A variety of indices have been developed, with the most popular indices being the IPS (Coste 1982), the IBD (Lenoir and Coste 1996; Prygiel and Coste 2000), the trophic diatom index (Kelly and Whitton 1995), and the Sladecek index (SLA) (Sladecek 1973). All of the above indices were developed based on a weighted average equation (Zelinka and Marvan 1961) in which the optima and tolerance values for each taxon were determined regardless of the reference conditions specific to each ecoregion or stream type. Species optima and tolerances are also generally derived from physico-chemical data, which implies that biomonitoring depends on environmental variables and leads to a circular argument. The main reason for using biota is precisely the complementary and nonredundant information provided on the ecosystem status. The most logical approach, in term of ecological integrity, is to derive bioindication information directly from community structure.

Based on the results of the present study, Lavoie et al. (2006) developed a diatom-based index that integrates different types of stream alterations and provides information related to the "distance" from the less-impacted state. The eastern Canadian diatom index (IDEC) used correspondence analysis (CA) to develop a "chemistry-free" index in which the position of the sites along the gradient of maximum variance (first axis) is strictly determined by diatom community structure and is therefore independent of measured environmental variables. The index value indicates the distance of each diatom community from its specific reference community. A high index value represents a non- or less-impacted site, whereas a low index value represents a more heavily impacted site. Two subindices were developed based on two sets of reference communities. The IDEC circumneutral subindex includes the sites that have reference communities characteristic of slightly acidic or neutral environments, whereas the IDEC alkaline subindex includes the sites that have reference communities characteristic of environments in which pH values are naturally higher than 7.65. The distinction between the two subindices is fundamental to ensuring that each stream has the potential to reach a high IDEC value following complete restoration of its ecosystem.

Development of a predictive model

The present work represents the preliminary phase in the

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elaboration of a model to predict the diatom community composition expected at a given site following restoration. To predict diatom community composition, predictive modelling assumes that a site is in its reference state. As stated by Reynoldson et al. (1997), if a test site can be associated with a group of reference sites representing the reference condition, then those reference sites can be used to predict community composition expected at the test site in the absence of disturbance. Stream and watershed attributes for groups of reference sites may be compared to identify a subset of variables used in the prediction of group membership. The RIVPACS predicts macroinvertebrate fauna at a given site from a small number of environmental parameters. By comparing the observed fauna with the predicted or "target" fauna, a measure of site integrity can be obtained (Wright et al. 2000). The AusRivAS is based on the RIVPACS model with the exception that major habitats are sampled and modelled separately. The BEAST (Reynoldson et al. 1995, 1997) is similar to the AusRivAS and RIVPACS approaches but uses abundances of macroinvertebrates instead of their presence or absence. Similar approaches were recently employed in Europe to predict the community structure of aquatic communities using advanced modelling techniques such as artificial neural networks, bayesian models, and genetic algorithms (Lek et al. 2005). From our results, it appears that a predictive model could be developed using, as predictors, the variables responsible for pH variations in reference streams, such as the proportion of gneiss–paragneiss, felsic, or clay rocks. These variables could be used to predict the group (circumneutral or alkaline community) to which impacted streams in southern Québec belong.

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Appendix A

Table A1. Description of the environmental variables.

Variables	Description	Units
AP	Appalachians	
CS	Canadian Shield	
SL	St. Lawrence Lowlands	
CHL	Chlorophyll *a*	mg·cm^{-3}
CON	Conductivity	µS·cm^{-1}
DOC	Dissolved organic carbon	mg·L^{-1} C
FC	Faecal coliforms	UFC 100 mL^{-1}
NH_3	Ammonia (N-NH_3)	mg·L^{-1} N
NO_3	Nitrates–nitrites	mg·L^{-1} N
O_2	Dissolved oxygen	mg·L^{-1}
pH	pH	pH
T	Temperature	°C
TN	Total nitrogen	mg·L^{-1} N
TP	Total phosphorus	mg·L^{-1} P
TUR	Turbidity	NTU (nephelometric turbidity units)
SRP	Soluble phosphorus	mg·L^{-1} P
SS	Suspended solids	mg·L^{-1}
VEL	Current velocity	m·s^{-1}
ALT	Altitude	m
AREA	Watershed area	km^2
DIST	Distance to source	km
EMBANK	Embankment	m
SUBS	Dominant substrate	Ordinal variable
WIDTH	Stream width	Ordinal variable
FOREST	Forested area	% of watershed
URBAN	Urban area	% of watershed
WATER	Water surface area	% of watershed
WETLAND	Wetlands + bogs	% of watershed
ALLU	Alluvium deposits	% of watershed
EOL	Eolian deposits	% of watershed
FLUVIO	Fluvioglacial deposits	% of watershed
LACU	Lacustrine deposits	% of watershed
MARIN	Marine deposits	% of watershed
ROCK	Surficial bedrock	% of watershed
TILL	Till deposits	% of watershed
GNEISS	Gneiss and paragneiss	% of watershed
CLAY	Clay rocks (e.g., mudrock and schist)	% of watershed
CARBON	Carbonated rocks (e.g., limestone, marble, and dolomite)	% of watershed
FELS	Felsic rocks (e.g., granite and tonalite)	% of watershed
INTER	Intermediate rocks (e.g., syenite)	% of watershed
MAF	Mafic rocks (e.g., basalt, diorite, and anorthosite)	% of watershed
SILI	Siliceous rocks (e.g., sandstone, arkose, and quartzite)	% of watershed
UMAF	Ultramafic rocks	% of watershed
ANIMAL	Animal population	Animal units
HERBIC	Area with herbicide use	% of watershed
MANURE	Area with manure use	% of watershed
PASTURE	Pasture area	% of watershed
POP	Human population	Number

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Patterns of hydrogen peroxide among lakes of the Mackenzie Delta, western Canadian Arctic

Catherine M. Febria, Lance F.W. Lesack, Jolie A.L. Gareis, and Max L. Bothwell

Abstract: Dissolved organic carbon (DOC) in Mackenzie Delta lakes varies in composition and concentration, ranging from low concentrations and high colour in frequently flooded lakes to high concentrations in clear lakes that are infrequently flooded. DOC is a precursor to the photochemical production of hydrogen peroxide (H_2O_2) in lake waters. Here we assessed the patterns of H_2O_2 using three approaches: (*i*) H_2O_2 levels were tracked in 40 lakes during the open water season from the Arctic summer solstice (24 h sunlight) to late summer; (*ii*) diurnal dynamics of in situ H_2O_2 were tracked in a pair of lakes with contrasting DOC regimes (coloured vs. noncoloured DOC); and (*iii*) buildup of H_2O_2 was tracked in experimental microcosms of lake water exposed to manipulated UV levels. H_2O_2 levels were highest at the solstice and in intermittently flooded lakes. During 24 h daylight, lakes with contrasting DOC regimes showed cumulative build up of H_2O_2 during multiple cloudless days. Cumulatively, H_2O_2 was highest in the Delta lake with a higher DOC concentration and low in colour. H_2O_2 buildup in lake water shielded from UVB exposure was not significantly lower from microcosms under full sunlight. UVA was the most important in coloured DOC photobleaching and H_2O_2 production.

Résumé : La composition et la concentration du carbone organique dissous (DOC) dans les lacs du delta du Mackenzie varient, allant de concentrations faibles et de colorations foncées dans les lacs à inondations fréquentes à des concentrations élevées dans les lacs clairs à inondations rares. DOC est un précurseur dans la production photochimique du peroxyde d'hydrogène (H_2O_2) dans les eaux de lac. Nous évaluons les patrons d'H_2O_2 d'après trois stratégies: (*i*) en suivant les concentrations d'H_2O_2 dans 40 lacs pendant la période d'eau libre depuis le solstice d'été de l'arctique (24 h d'éclairement) à la fin de l'été; (*ii*) en déterminant la dynamique journalière d'H_2O_2 in situ dans deux lacs à régimes de DOC différents (DOC incolore et DOC coloré) et (*iii*) en mesurant l'accumulation d'H_2O_2 dans des microcosmes expérimentaux d'eau de lac exposés à des niveaux variés d'UV. Les concentrations d'H_2O_2 sont maximales au solstice et dans les lacs inondés de façon intermittente. Durant l'éclairement de 24 h, les lacs possédant des régimes différents de DOC subissent une accumulation d'H_2O_2 lorsqu'il y a plusieurs jours de suite sans nuages. De façon cumulative, H_2O_2 atteint un maximum dans le lac du delta qui a une plus forte concentration de DOC et une couleur pâle. L'accumulation d'H_2O_2 dans l'eau de lac protégée de l'exposition à l'UVB n'est pas significativement plus basse que celle des microcosmes en plein soleil. L'UVA joue le rôle primordial dans le photo-blanchiment du DOC coloré et la production d'H_2O_2.

[Traduit par la Rédaction]

Introduction

This study examined the patterns of photochemical production of hydrogen peroxide (H_2O_2) among Arctic lakes in the Mackenzie Delta. Mackenzie Delta lakes are expected to experience a shift in flood frequency and magnitude because of climate change (Marsh and Lesack 1996; Rouse et al. 1997; Lesack et al. 1998). Simultaneously, stratospheric ozone loss in the circumpolar Arctic is predicted to increase (Shindell et al. 1998), which will result in increased levels of ultraviolet radiation (UVR) at the earth's surface. Therefore, while the potential for increased photochemical activity at Arctic latitudes is high, it is not well understood (Zepp et al. 2003). Our study represents the first among all studies of circumpolar Arctic river deltas to assess patterns of H_2O_2 produced by interactions of solar radiation and dissolved organic carbon in surface waters.

Dissolved organic carbon (DOC) plays an important role in the optical properties, energy cycling, and photochemical processes in surface waters (Lindell et al. 1996;

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Obernosterer and Herndl 2000; Pienitz and Vincent 2000). The Mackenzie Delta lakes, as a function of topographic position (Marsh and Hey 1989), vary considerably in water transparency, water color, macrophyte abundance, zooplankton composition, and in the concentration and quality of DOC (Lesack et al. 1998; Squires and Lesack 2003*b*; Spears and Lesack 2006). Delta lakes contain both allochthonous, coloured DOC (C-DOC) and autochthonous, noncoloured DOC (NC-DOC) in relation to their position along the flood-frequency gradient, ranging in total DOC (T-DOC) concentrations from 6 to 18 mg·L^{-1} (Lesack et al. 1991; Spears and Lesack 2006). It is critical to this study system to distinguish the relative contribution of both C-DOC and NC-DOC to T-DOC among all Mackenzie Delta lakes. Both T-DOC and C-DOC are measurable parameters, and the difference between the two concentrations is used to infer the amount of NC-DOC present in each lake. Overall, gradients of T-DOC and C-DOC run counter to one another, whereby frequently flooded lakes are high in C-DOC but low in T-DOC, in contrast with infrequently flooded lakes that are low in C-DOC but high in T-DOC (Teichreb 1999; Squires 2002; Spears and Lesack 2006).

River flooding in the Delta is critical in providing water and nutrients, including DOC, to Delta lakes from the surrounding watershed. Although flooding ought to decrease because of climate change, C-DOC inputs are likely to increase as temperatures increase and permafrost melts (Rouse et al. 1997; Perin and Lean 2004), leading to changes in underwater spectral irradiance (Pienitz and Vincent 2000). Because one primary function of C-DOC is to attenuate UVR in the water column, increased C-DOC levels will provide an additional "sunscreen" for aquatic organisms lacking UVR-screening pigments (Laurion et al. 1997; Perin and Lean 2004). Simultaneously, DOC–UVR interactions may also increase and produce additional biologically toxic free radicals, including superoxides, hydroperoxides, and H_2O_2 (Cooper et al. 1989).

H_2O_2 is longer-lived than other photoproducts and is thought to be a reasonable proxy for photochemical activity in fresh waters (Cooper et al. 1988) and therefore was the photoproduct of interest in our study. Both ultraviolet-A (UVA, 320–400 nm) and ultraviolet-B (UVB, 280–320 nm) radiation are effective in catalyzing photochemical reactions with DOC; however, because of the sheer abundance of UVA photons, UVA can be more critical in H_2O_2 formation (Scully and Vincent 1997; Jerome and Bukata 1998) and is perhaps the more efficient type of light for H_2O_2 production (Wong and Wong 2001). Regardless of how it is produced, H_2O_2 can be toxic to bacteria (Xenopoulos and Bird 1997) and is known to react strongly with other free radicals and metals in the water column (Zepp et al. 1992). In fresh waters, H_2O_2 has been shown to exhibit diurnal variability, with peak concentrations occurring during the day and near-complete decay occurring at night (Cooper and Lean 1989; Scully and Vincent 1997). Only a few studies have examined H_2O_2 levels in Arctic or sub-Arctic lakes (Scully et al. 1996; Scully and Vincent 1997). Scully and Vincent (1997) suggested that H_2O_2 production could be considerable in high-latitude systems, where solar day lengths may be up to 24 h. Scully et al. (1996) reported that high Arctic lakes with characteristically low DOC concentrations and low water color also tend to have comparatively low H_2O_2 concentrations. By contrast, boreal lakes characterized by highly stained waters can have high photochemical activity and H_2O_2 production (Cooper and Lean 1989; Scully et al. 1996; Häkkinnen et al. 2004). This study sought to determine whether the flood-frequency and DOC gradients in the Mackenzie Delta lakes would be reflected in a pattern in DOC–UVR interactions. We are not aware of any studies that have examined patterns of H_2O_2 among the productive and lake-rich river deltas in the circumpolar Arctic. Overall, we expected H_2O_2 levels to be influenced by the strong variation in DOC composition among lakes of the Mackenzie Delta and the 24 h daylight associated with the Arctic summer. We tested the following hypotheses: (*i*) H_2O_2 levels will be highest in lakes with the highest levels of C-DOC; (*ii*) H_2O_2 levels will be highest around the solstice and lower later in the summer; (*iii*) H_2O_2 levels will progressively build up during sequences of cloudless days with 24 h daylight; and (*iv*) H_2O_2 buildup will be highest where the optimal combination of C-DOC and UVR (i.e., both UVA and UVB) exposure is present. We achieved this by sampling 40 lakes of varying flooding frequency and DOC regime three times during the open-water season, tracking diurnal concentrations of H_2O_2 in a pair of lakes with contrasting DOC regimes, and tracking the hourly buildup of H_2O_2 in microcosms of lake water where exposure to UVB and UVA irradiance was experimentally manipulated. Herein, we report the first results on patterns of in situ H_2O_2 among delta lakes and the potential roles of DOC and UVR in controlling the pattern.

Materials and methods

Study site

The Mackenzie River is a north-flowing river that passes through a lake-rich delta containing approximately 49 000 lakes (Emmerton 2006) before entering the Beaufort Sea (Fig. 1). The Mackenzie River plus all adjacent lakes and channels are ice-covered for a large portion of the year. Extensive annual ice jamming in the river in late May to early June is primarily responsible for the magnitude of flooding in the Delta each spring, which replenishes the lakes with floodwater. Mackenzie Delta lakes are generally quite shallow (mean depths typically <2 m, maximum depths <4 m), small (<10 ha), and well mixed to their bottom (MacKay 1963). In the central Delta, the majority of the lakes become disconnected from the main channel during the summer months, experiencing a negative water balance owing to low levels of precipitation and high rates of evaporation (Marsh and Hey 1989).

Lakes can be differentiated from each other based on their sill elevation, which is defined as the highest elevation along the channel thalweg connecting the lake to the distributary channel (Marsh and Hey 1989). Delta lakes represent a flooding gradient with the greatest riverine influence occurring at the lowest sill elevations (highest flooding frequencies). Marsh and Hey (1989) classified lakes of the Delta as no, low, or high closure (adapted from MacKay 1963) based on whether lakes exceeded the 1-year return period of flooding with river water (high closure) or exceeded the 1-year return period in becoming isolated from the river at some

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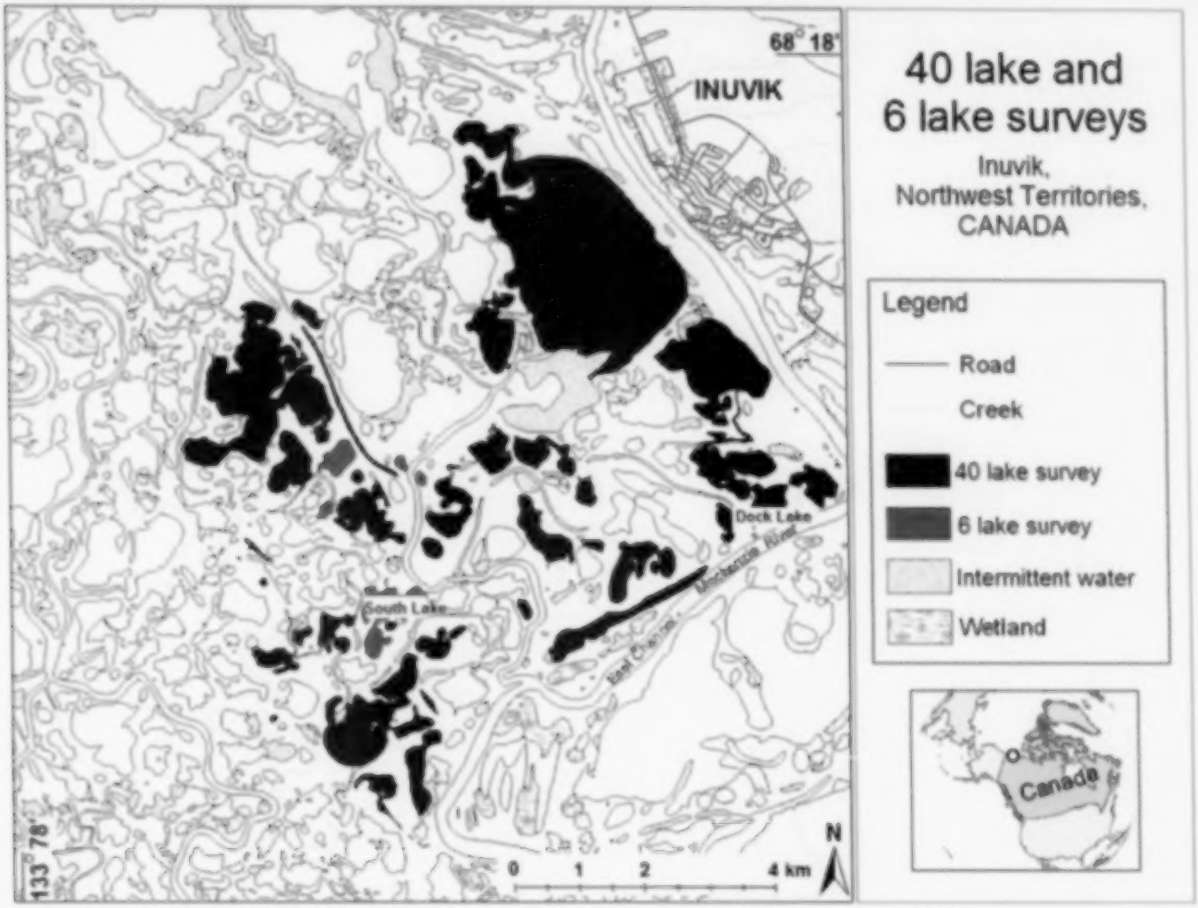
Fig. 1. Map showing 40-lake subsystem study area in the Mackenzie Delta, western Canadian Arctic.



point during the year (no closure). In this study, the no, low, and high closure lakes are referred to as frequently, intermediately, and infrequently flooded lakes, respectively. Numerous limnological properties are strongly related to this flood-frequency gradient, including inorganic nutrients (Lesack et al. 1991, 1998), water transparency and suspended sediments (Squires and Lesack 2003*a*), and C-DOC and DOC concentrations (Spears and Lesack 2006). The lakes studied in this investigation are all located near the town of Inuvik (68°19′N, 133°29′W) on the east channel of the Mackenzie Delta.

Water sampling and chemical analyses

Lakes were generally sampled from a central location, and water was collected from just below the surface. H_2O_2 samples were collected in 60 mL amber glass vials with a Teflon™ cap, and samples for DOC were collected in new Nalgene™ high-density polyethylene bottles. Samples were placed in a darkened cooler until processing at the Inuvik Research Centre (Inuvik, Northwest Territories). H_2O_2 and C-DOC measurements were carried out immediately upon return to the Inuvik lab, whereas DOC samples were filtered, frozen, and analyzed within a few months at Simon Fraser University (Burnaby, British Columbia).

H_2O_2 was measured using the horseradish peroxidase scopoletin method (Holm et al. 1987) on a Turner Designs Inc. (Sunnyvale, California) model 111 fluorometer with a near-UV lamp and a 365 nm excitation filter (Corning 7-60) plus 490 nm emission (Turner 2A and 65A) filter combination. The concentration of H_2O_2 was determined by the method of standard additions (Larsen et al. 1973) using three known H_2O_2 spikes for each individual lake, effectively creating a standard curve for each lake. Duplicate aliquots from each sample were analyzed, and samples were remeasured every few hours to track decay with time. Replicate analyses for the same concentration were within 10% for concentrations >50 nmol·L^{-1}. Values reported here are the mean concentrations of the duplicates for each sample. Samples for H_2O_2 were taken by subsurface grabs at mid-lake stations. Cognizant that H_2O_2 can be quite dynamic in lake waters, our single point measurements from lakes in the Delta are reasonable estimates of H_2O_2 concentrations, because the lakes are shallow and well mixed, and because the methodologies were very consistent (all samples taken from mid-

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lake sampling points and subsurface grabs were taken near solar noon) and designed to address the time sensitivity of the analyses.

Measurement of the colored or humic-derived fraction of DOC (C-DOC) was done spectrophotometrically (Milton Roy Spectronic 501, Milton Roy Company, Ivyland, Pennsylvania) by measuring the optical absorbance of 0.22 µm (Millipore polycarbonate membrane, Millipore Corp., Bedford, Massachusetts) filtered water samples in a 10 cm quartz cuvette and using distilled–deionized water as a reference. Absorbances at 330 nm were corrected for background scatter by subtracting absorbance at 740 nm. Scatter-corrected absorbances at 330 nm were subsequently converted to an attenuation coefficient (Ka330) to facilitate comparisons with other C-DOC measurements. Following the convention of Scully and Lean (1994), the absorption measurements reported in this paper were transformed into natural logs and standardized per metre pathlength and is analogous to an attenuation coefficient. We report the index Ka330 as a direct measure of C-DOC in this paper and for consistency with previously published values for Mackenzie Delta lakes.

To determine T-DOC concentrations, water samples were filtered through a Whatman GF/C filter (Whatman Inc., Clifton, New Jersey) and kept frozen until analysis. Prior testing of GF/C filtration versus filters with smaller pore sizes established that the C content of bacteria-size particles passing through GF/C filters, but not through 0.22 µm polycarbonate filters, was an unimportant component of the relatively high levels of T-DOC in these lakes. A subset of samples were reanalyzed after refiltering through a Whatman GF/F filter, and the effect of the smaller GF/F matrix on DOC results was found to be negligible in this case. T-DOC was measured by first sparging dissolved inorganic carbon (DIC) from an aliquot of sample, then combusting the aliquot to CO_2 via the high-temperature catalytic oxidation method and subsequently measuring the CO_2 with a nondispersive infrared detector (Shimadzu TOC-V series analyzer, Shimadzu Corp., Kyoto, Japan). Mackenzie Delta water is typically high in DIC, and care was taken to ensure the sparging step was fully adequate. Both GF/C and polycarbonate filters were well rinsed with analytical-grade deionized water prior to sample filtration.

Surveys of the 40-lake subsystem

Extended surveys of H_2O_2 patterns among lakes over the open-water season were performed on a set of 40 spatially discrete lakes that were chosen on merit of sill elevation and historical DOC composition, representing a wide range for both parameters. The set of lakes was surveyed three times each on 8 July, 26 July, and 14 August 2004. Samples were collected within an hour of solar noon when photochemical activity was expected to be highest (1500 local time) and were transported immediately to the Inuvik Research Centre for processing.

Diurnal H_2O_2 dynamics in two lakes

The diurnal dynamics of H_2O_2 formation were tracked during a period of cloudless weather in two contrasting lakes, Dock Lake and South Lake, to assess the effect of 24 h daylight near the summer Arctic solstice. These lakes are reasonable endpoints in the typical Delta DOC gradient, with Dock Lake representing a low C-DOC and high T-DOC regime versus South Lake, which represents a high C-DOC and low T-DOC regime. Dock Lake had not been flooded by river water for several years prior to this study (L. Lesack, unpublished data) and is further characterized by a high sill elevation, relatively high water transparency, abundant macrophytes, and DOC levels typically around 18 $mg \cdot L^{-1}$. By contrast, South Lake typically remains connected to the river channel throughout the open-water season. It is further characterized by low water transparency and DOC levels typically around 7 $mg \cdot L^{-1}$ or less. Both lakes were sampled every 4 h for a 40 h period near the 2004 solstice, with Dock Lake being tracked during the period 26–28 June 2004 and South Lake tracked during 2–4 July 2004. To ensure comparable levels of irradiance between the two lakes during the periods of observation, photosynthetically active radiation (PAR = 400–700 nm) was measured on site at the lakes each time water samples were taken while incoming solar radiation was continuously tracked at the Inuvik Research Centre (measured as $W \cdot m^{-2}$). PAR was measured just beneath the water surface using an upward-facing LI-COR 2-pi underwater sensor (LI-195A, LI-COR Biotechnology, Lincoln, Nebraska).

H_2O_2 buildup with and without UVR

To further assess the comparative buildup of H_2O_2 between Dock Lake and South Lake, H_2O_2 levels were tracked in replicated microcosms of lake water exposed to natural sunlight with and without UVB and UVB+UVA removed by filters. This experimental approach ensured that differences in daily irradiance or water renewal rates among the lakes that would potentially affect in situ observations were not factors. This design also assessed the role of chromophoric C-DOC substrate availability in the build-up of H_2O_2 levels during exposure to varying natural light over a 10–14 h period. The experiment was conducted twice, once under cloudy conditions (5 July 2004) and again under cloudless conditions (9 July 2004). Prior to each experiment, water was collected from each lake and returned to the Inuvik Research Centre. Water was collected as an integrated sample of the upper water column using a 1 m long plastic tube sampler (67 mm internal diameter) and was transferred to a plastic bucket until 10 L of water was obtained. Lake water was then transferred to a rinsed, darkened jerry can (10 L) and placed in a cooler during transport to the Inuvik Research Centre. Triplicate 1 L microcosms were filled with well-mixed, unfiltered lake water and placed in a water-filled (for temperature control) outdoor incubator, under one of the three irradiance treatments. The microcosms were irradiated in the incubator for 10 h during the 5 July experiment (cloudy conditions) and for 13.5 h during the 9 July experiment (cloudless conditions). H_2O_2 concentrations, DOC, and C-DOC were measured at the start of the experiment (0 h). Measurements were then repeated immediately following the incubation period. Total incoming solar radiation ($W \cdot m^{-2}$) was continuously logged at the Inuvik Research Centre, and PAR irradiance (LI-COR sensor), averaged over 0.5 h intervals just beneath the water surface in the incubator, was logged (LI-1000 data logger, LI-COR Biotechnology) during the two experiments.

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The microcosms were cylindrical vessels (1 L volume), fabricated by ASI Plastics (Coquitlam, British Columbia) out of Acrylite™ OP-4 (GE Polymershape, Burlington, Ontario), and sealed with a rubber stopper inserted into the bottom of the vessel. Acrylite™ OP-4 passes approximately 90% of the visible and UVA irradiance and about 70% of UVB irradiance through the walls of the microcosm (M. Bothwell, unpublished data). The incubator was 1.2 m wide × 2.4 m long and deep enough (i.e., contained enough water) to submerge the sides of the microcosms, with the tops (i.e., OP-4 end panel) slightly above the water level. Sections of the incubator were then screened to selectively remove wavelengths from the full solar irradiance. UVB wavelengths were removed using Mylar-D™ sheeting (4 mil, GE Polymershape, Burlington, Ontario; hereafter referred to as Mylar). UVA+UVB wavelengths were removed (leaving PAR wavelengths only) using Acrylite™ OP-2 plexiglass sheeting (Cadillac Plastics, Coquitlam, British Columbia). As a methodological control, polyethylene sheeting was used for full sunlight treatments, which effectively allows the passage of full spectrum sunlight (UVB+UVA+PAR).

Similar to other studies on the effects of UVR on shallow, freshwater systems, plastic filters were used to remove broadbands of UVR to determine the impacts of selected regions in the UVR range (Bothwell et al. 1994; Donahue 2000; Kelly et al. 2003). The three irradiance treatments we used were as follows: (*i*) UVB+UVA+PAR (i.e., full solar irradiance = control), (*ii*) UVA+PAR (Mylar filter), and (*iii*) PAR only (OP-2 filter). The radiation transmission properties of each filter used were confirmed in the field via radiation scans (data available in Febria 2005) on a cloudless day shortly after the experiments using an OL-754 Portable UV-visible spectroradiometer (Optronics Laboratories, Inc. Orlando, Florida).

Statistical analyses

Pearson's correlation coefficients were calculated to test for any significant relationship between T-DOC and sill elevation and between C-DOC and sill elevation ($\alpha = 0.05$). To test for significance between seasonal sampling of H_2O_2, data was normally distributed, and differences between sampling dates (8 July, 26 July, and 14 August) were tested using a one-way analysis of variance (ANOVA). For the microcosm experiments, H_2O_2 and C-DOC data were normally distributed following a log transformation and tested for significance using a one-way ANOVA followed by comparison for all pairs using Tukey's honestly significant difference (HSD). Statistical analysis for this experiment included testing for differences between (*i*) experiments (i.e., cloudy and cloudless) and (*ii*) lakes (i.e., South Lake and Dock Lake) and (*iii*) among UV treatments (i.e., PAR only, PAR+UVA, PAR+UVA+UVB, and 0 hour reference). All statistical analyses were conducted using JMP statistical software (Version 5.0, SAS Institute Inc.). Levels of significance were set at $\alpha = 0.05$, and the experimental design was conducted according to Sokal and Rohlf (1995).

Results

DOC gradients among the lakes

The DOC gradients in the Mackenzie Delta vary among years, and the gradients observed during 2004 are important for interpreting our hypotheses about patterns of H_2O_2 among the lakes. Although variable from year to year, T-DOC among the lakes typically demonstrate defined counter gradients of C-DOC and NC-DOC, where C-DOC measurements decline with increasing sill elevation (decreasing flood frequency), while T-DOC measurements increase (Lesack et al. 1991; Febria 2005; Spears and Lesack 2006). As expected, in July 2004, T-DOC was positively correlated with sill elevation among the 40 lakes ($r = 0.3862$, $p = 0.0152$). In contrast, a negative relationship between C-DOC and sill elevation among the 40 lakes was more subtle ($r = -0.900$, $p = 0.5857$) than measurements made in previous years (Spears and Lesack 2006; Teichreb 1999). C-DOC represents the abundance of chromophoric DOC molecules during the early open-water period, prior to considerable photobleaching, that should participate in photochemical production of H_2O_2 among the lakes. If we convert the Ka330 index to an analogous value at 310 nm using an empirical relationship between absorbance at 330 nm versus at 310 nm in Delta lakes, an approximate estimate of C-DOC concentration in $mg \cdot L^{-1}$ can be obtained from the regression between Ka310 and measured DOC concentration published by Scully and Lean (1994, assumes C-DOC = T-DOC). Based on this approximation, the C-DOC in Fig. 2 would range between about 5.6 and 9.4 $mg \cdot L^{-1}$. In comparison with the range of T-DOC concentrations we actually measured in these lakes (~5–16 $mg \cdot L^{-1}$), the estimated C-DOC concen-

Fig. 2. Relationships between (*a*) colored dissolved organic carbon (C-DOC index expressed as Ka330) and sill elevation (in metres above sea level, m asl) and (*b*) total dissolved organic carbon (T-DOC) and sill elevation for the 40-lake subsystem on 8 July 2004. Pearson's correlation coefficients are reported for each relationship.

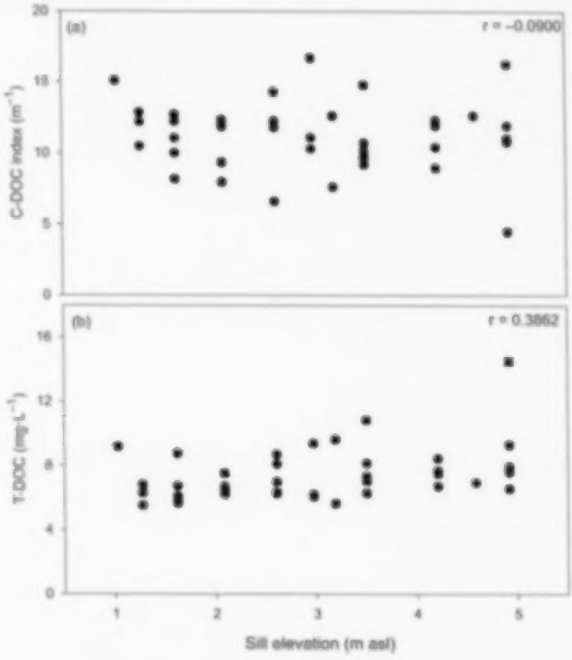

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Fig. 3. Seasonal variation in H_2O_2 with colored dissolved organic carbon levels (C-DOC index expressed as Ka330) on (*a*) 8 July, (*b*) 26 July, and (*c*) 14 August in the 40-lake subsystem over the open-water period of 2004.

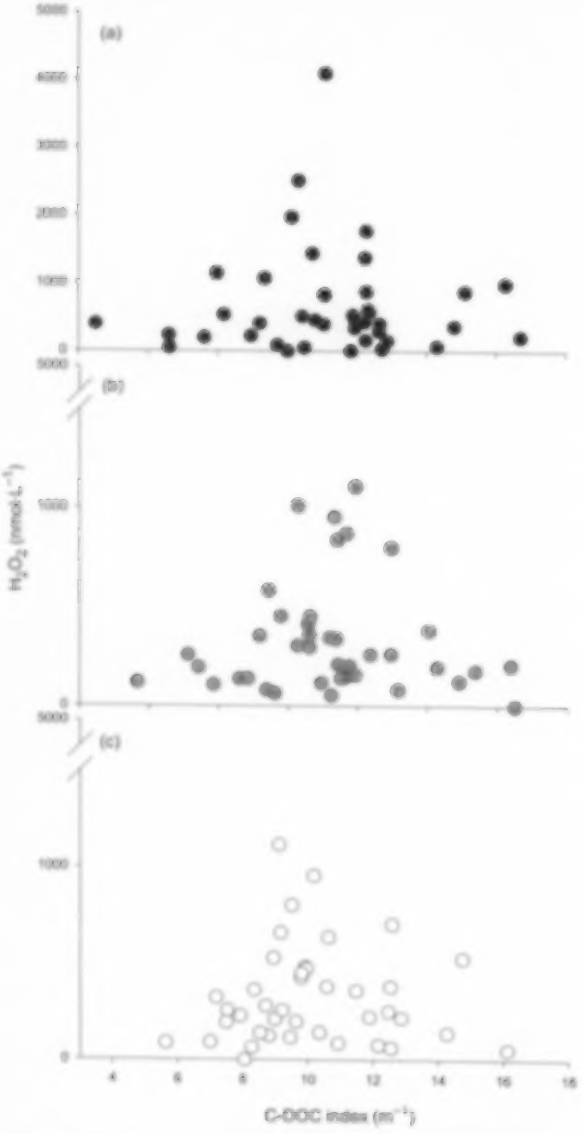
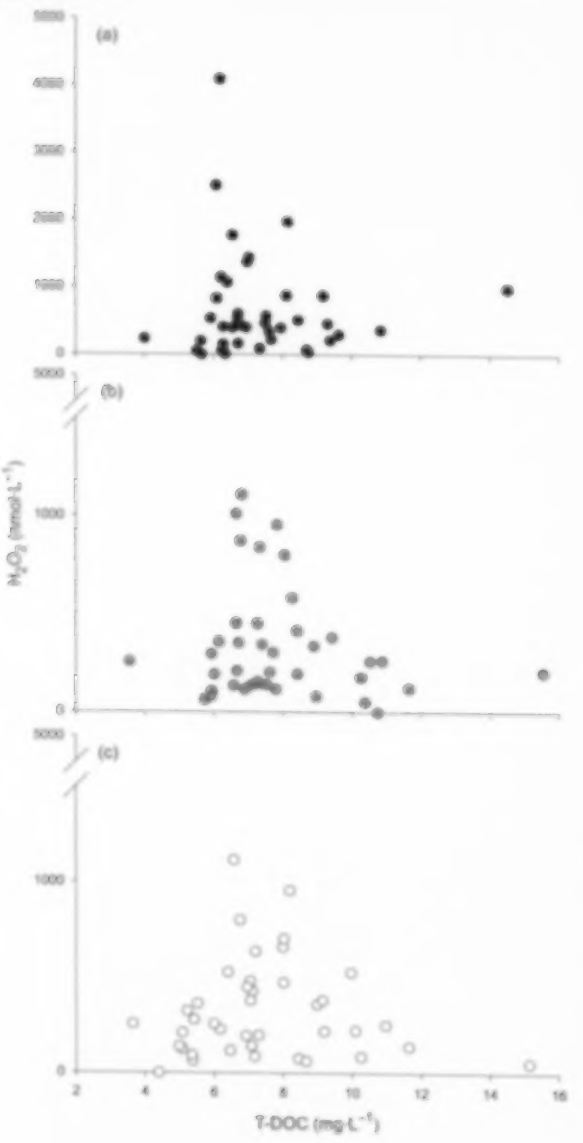


Fig. 4. Seasonal variation in H_2O_2 with total dissolved organic carbon concentrations (T-DOC) on (*a*) 8 July, (*b*) 26 July, and (*c*) 14 August in the 40-lake subsystem over the open-water period of 2004.



trations indicate that the inferred NC-DOC concentrations were as much as 50% of T-DOC in the high sill elevation lakes, even during the early open-water period.

Seasonal H_2O_2 pattern

The results also show that H_2O_2 levels varied considerably in the summer, with peak levels observed on 8 July followed by considerably lower H_2O_2 levels observed in subsequent surveys (27 July and 14 August). A one-way ANOVA confirmed that H_2O_2 levels were significantly different among the three dates ($F = 6.2714$, $p = 0.0026$). The highest level observed at an individual lake was 4100 nmol·L^{-1}, occurring early in the season (8 July), with overall H_2O_2 levels dropping dramatically by the end of July and leveling out in August. This strong seasonal variation in photoproduction of H_2O_2 that is enhanced early in the open-water season may be facilitated by a combination of abundant, nonphotobleached chromophoric DOC present after ice-out, particularly long day lengths, and a favourable sun angle associated with the summer solstice.

The highest concentrations of H_2O_2 were found among lakes with intermediate levels of both C-DOC and T-DOC (Figs. 3 and 4). This was contrary to our expectation that

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Fig. 5. Diurnal variations in H_2O_2 concentration (solid line) and photosynthetically active radiation (PAR; shaded, broken line) at (*a*) Dock Lake from 26 to 28 June (T-DOC = 17.25 mg·L^{-1}) and (*b*) South Lake from 2 to 4 July (T-DOC = 7.78 mg·L^{-1}).

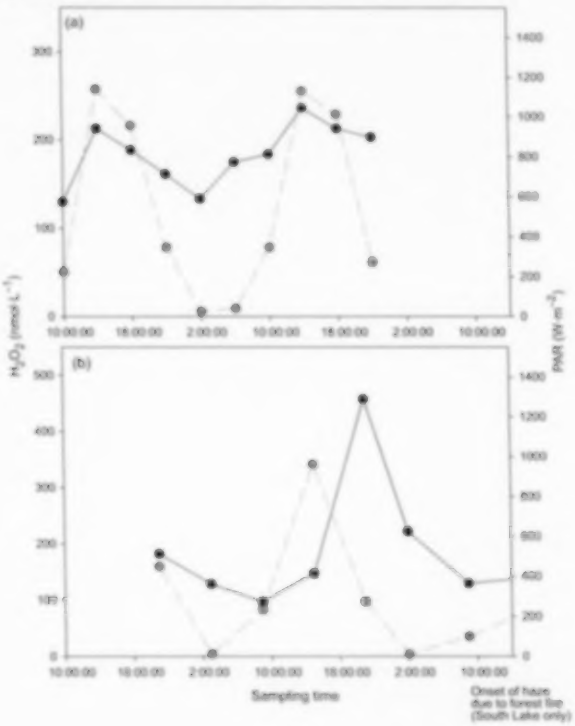
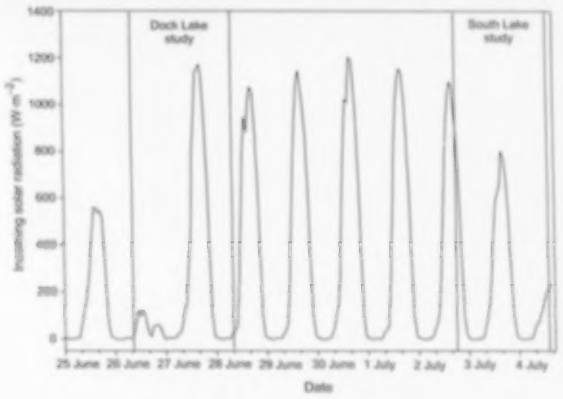


Fig. 6. Diurnal variation in incoming solar radiation (W·m^{-2}), as measured at the Inuvik Research Centre from 25 June to 4 July.


Dock Lake study
South Lake study
Incoming solar radiation (W·m^{-2})
25 June 26 June 27 June 28 June 29 June 30 June 1 July 2 July 3 July 4 July
Date

H_2O_2 levels would be highest where C-DOC is highest. The results suggest that optimal conditions for photoproduction of H_2O_2 may occur where there is a trade-off between photochemical substrate, such as chromophoric macromolecules of DOC (high in lakes with low sill elevations), and the amount of UVR penetrating through the water column (high in lakes with high sill elevations). Hence, the highest H_2O_2 concentrations may be found in lakes with intermediate sill elevations.

We examined other possible factors that could be related to H_2O_2 buildup in lake waters, including T-DOC concentration (Fig. 4), and performed multiple regression analyses with other abiotic variables (Febria 2005). Potential relationships between H_2O_2 and lake area and between H_2O_2 and average lake depth (variables potentially related to lake mixing rates) were assessed for all three surveys and were not statistically significant (not shown here; Febria 2005).

Diurnal dynamics of H_2O_2

Clear diurnal patterns in H_2O_2 concentrations were revealed in both Dock Lake and South Lake. As expected, H_2O_2 levels generally followed, but lagged slightly behind, solar intensity (Fig. 5). Peaks in H_2O_2 concentrations roughly corresponded to solar noon (~1500) and never completely declined to zero. H_2O_2 concentrations were generally lower and fluctuated less in Dock Lake during the period of observation (between 150–250 nmol·L^{-1}) than in South Lake (between 100–500 nmol·L^{-1}). The difference between the lakes was at least partly because of differences in the pattern of solar radiation, particularly the antecedent conditions prior to each set of observations (Fig. 6). The Dock Lake observations were preceded by cloudy weather that carried over to the beginning of the observation period. In the case of South Lake, observations were preceded by 6 days of relatively cloudless weather, then during the latter part of the observation period, solar irradiance levels were moderately reduced by atmospheric haze from a forest fire to the south.

The potential for H_2O_2 buildup was evident in both lakes. Water samples taken at the same time of the day were consistently higher during day 2 of the observation periods. Additionally, H_2O_2 concentrations in Dock Lake 4 days after the diurnal observations were substantially higher (500 nmol·L^{-1}, data not shown) than measured previously. This high value of H_2O_2 from Dock Lake was made at the same time as measurements made on day 1 of the South Lake diurnal observation period and indicate that Dock Lake had greater H_2O_2 concentrations than South Lake at that time. Overall, H_2O_2 did appear to build up in both Delta lakes under cloudless conditions.

UVR and C-DOC effects on H_2O_2 buildup

We predicted that H_2O_2 would attain greatest concentrations where the combination of C-DOC substrate and UVB exposure was highest and that C-DOC photobleaching would be lowest under cloudy conditions. When water samples from Dock Lake and South Lake were irradiated in microcosms, Dock Lake had the most optimal combination of DOC composition and UVR exposure. For the cloudy experiment (5 July), significant differences were observed between lakes for both hourly production of H_2O_2 and C-DOC levels ($p = 0.0028$ and $p < 0.0001$, respectively; Table 1). For the experiment under cloudless conditions (9 July), significant differences were found for hourly H_2O_2 production and C-DOC levels between treatments ($p < 0.0001$ and $p = 0.0401$, respectively; Table 1).

Each experiment was tested individually and differences in H_2O_2 production and C-DOC levels among all treatments were evaluated. As expected, hourly H_2O_2 production levels were highest and apparent losses in C-DOC absorbance at 330 nm (i.e., C-DOC photobleaching) were greatest under

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Table 1. One-way analysis of variance (ANOVA) results that tested for differences in H_2O_2 production and coloured dissolved organic carbon (C-DOC) levels in experiments conducted under cloudy and cloudless conditions for both lakes ($\alpha = 0.05$).

	Cloudy		Cloudless	
Factor	F	p	F	p
$H_2O_2 \cdot h^{-1}$ × lake	12.674	0.003	2.100	0.161
C-DOC × lake	419.588	<0.0001	34.275	<0.0001
$H_2O_2 \cdot h^{-1}$ × treatment	3.850	0.025	20.611	<0.0001
C-DOC × treatment	0.288	0.833	3.335	0.037

the UVB+UVA+PAR (i.e., full sunlight) in both lakes (Fig. 7). H_2O_2 production and apparent C-DOC declines under UVB+UVA+PAR treatment were not significantly greater than the UVA+PAR treatment (Table 2). However, when all incident UVR was removed from the spectrum (i.e., PAR alone), H_2O_2 production and C-DOC levels were similar (and in some cases statistically nonsignificant) to the values observed at 0 h (i.e., $t = 0$ Ref). These results indicate that UVA was the most critical waveband to photochemical processes in the Mackenzie Delta lakes. Although biologically significant, UVB and PAR played only minor roles.

Both H_2O_2 production and C-DOC decline were found to be significantly greater in Dock Lake than in South Lake (Table 2). When comparing lakes, levels of H_2O_2 production were consistently higher in the Dock Lake microcosms than in South Lake microcosms. A similar trend was apparent for C-DOC photobleaching, where the greatest decline was observed in Dock Lake microcosms. This demonstrates the potential trade-off among photobleaching substrate, T-DOC concentration, and UVR penetration. Photochemical activity may be better facilitated in lakes such as Dock Lake (lower C-DOC, higher T-DOC, high sill elevation) versus South Lake (higher C-DOC, lower T-DOC, low sill elevation).

Discussion

Overall, the H_2O_2 values reported in this study compare reasonably with values reported by Scully et al. (1996) along a gradient in DOC and latitude from 43° to 75°N. However, some lakes in the Mackenzie Delta had much higher DOC and H_2O_2 concentrations than reported for other high Arctic lakes. The unique physical and geographical characteristics of the Mackenzie Delta lakes themselves may explain this anomaly. The lakes examined by Scully et al. (1996), while also in the high Arctic, were predominantly characterized by low DOC as a result of little vegetation in their surrounding catchments. In contrast, Mackenzie Delta lakes are floodplain lakes that can be high in DOC concentration and are strongly influenced by DOC inputs from regional river water, abundant macrophyte communities, and snowmelt runoff in local catchments of the lakes. H_2O_2 patterns and concentrations in lakes of the Delta expand the base of values reported thus far in the scientific literature.

Consistent with other studies (Jerome and Bukata 1998; Wong and Wong 2001), our experimental results indicate that radiation in the UVA is the most active waveband in the production of H_2O_2 in the lakes. This is likely due to the sheer number of photons that are present at this waveband. Thus, although much focus has been placed on the biological importance of the UVB portion of the solar spectrum, the indirect effects that H_2O_2 and other free radicals might have on the microbiological communities present deserve more attention in the future.

Seasonal H_2O_2 pattern and the Arctic summer

Few studies have tracked the seasonal dynamics of H_2O_2 in aquatic ecosystems. We are not aware of any study that has examined H_2O_2 dynamics in a system such as the Mackenzie Delta that consists of a diverse set of lakes with high DOC levels exposed to 24 h solar irradiance for an extended period around the Arctic summer solstice. For most of the year, lakes in the Delta are ice-covered and in the dark. However, at ice-out in the spring, regional runoff and local snowmelt provide large inputs of C-DOC to the lakes (Squires and Lesack 2003*b*). At the same time, the solar flux is at an annual maximum, and the sun is above the horizon continuously for many weeks. At that time of year, our study shows that both H_2O_2 and C-DOC in lake waters reach their highest levels, with peak H_2O_2 values of over 4000 nmol·L^{-1} near the solstice, falling to levels below 1000 nmol·L^{-1} in the late July and mid-August. The factors contributing to H_2O_2 production appear to dampen as DOC photobleaches and the Arctic daylight lessens, thereby reducing photochemical activity.

H_2O_2 values of 4000 nmol·L^{-1} are extremely rare for freshwater ecosystems, especially for Arctic waters. Concentrations can be as high as 25 000 nmol·L^{-1} (Blough and Zepp 1995), but most studies report values below 1000 nmol·L^{-1} in fresh and marine waters (Scully et al. 1996; Yocis et al. 2000; Obernosterer et al. 2001), with up to 3000 nmol·L^{-1} reported for some temperate latitude lakes (Scully et al. 1996). The observed levels are plausible given the known DOC conditions among Mackenzie Delta lakes, particularly early in the season when the peak levels were observed.

H_2O_2 diurnal dynamics and buildup

Our observations of diurnal dynamics show that the pattern of H_2O_2 formation that follows solar intensity corroborates other diurnal studies (Cooper and Lean 1989; Scully et al. 1995; Wilson et al. 2000), where H_2O_2 production responds to irradiance (PAR and UVR fluxes) with peak concentrations occurring up to a few hours after the peak irradiance flux. However, the pattern in our study was also affected by antecedent irradiance conditions, where extended sequences of cloudless weather lead to cumulative buildup of H_2O_2 levels in the lakes. Our evidence suggests that the continuous solar irradiance of the Arctic summer creates the opportunity for H_2O_2 to build up during cloudless weather, but the window of time when this can occur may be relatively short, as overall H_2O_2 levels among the lakes appear to decline significantly within a few weeks of the solstice.

H_2O_2 levels versus the DOC gradient

One of our initial hypotheses (i.e., H_2O_2 levels would be highest in lakes with the highest levels of C-DOC) was not supported by the data. The diurnal dynamics observations and the results of the microcosm experiments indicated that H_2O_2 concentrations would be higher in lakes with lower C-

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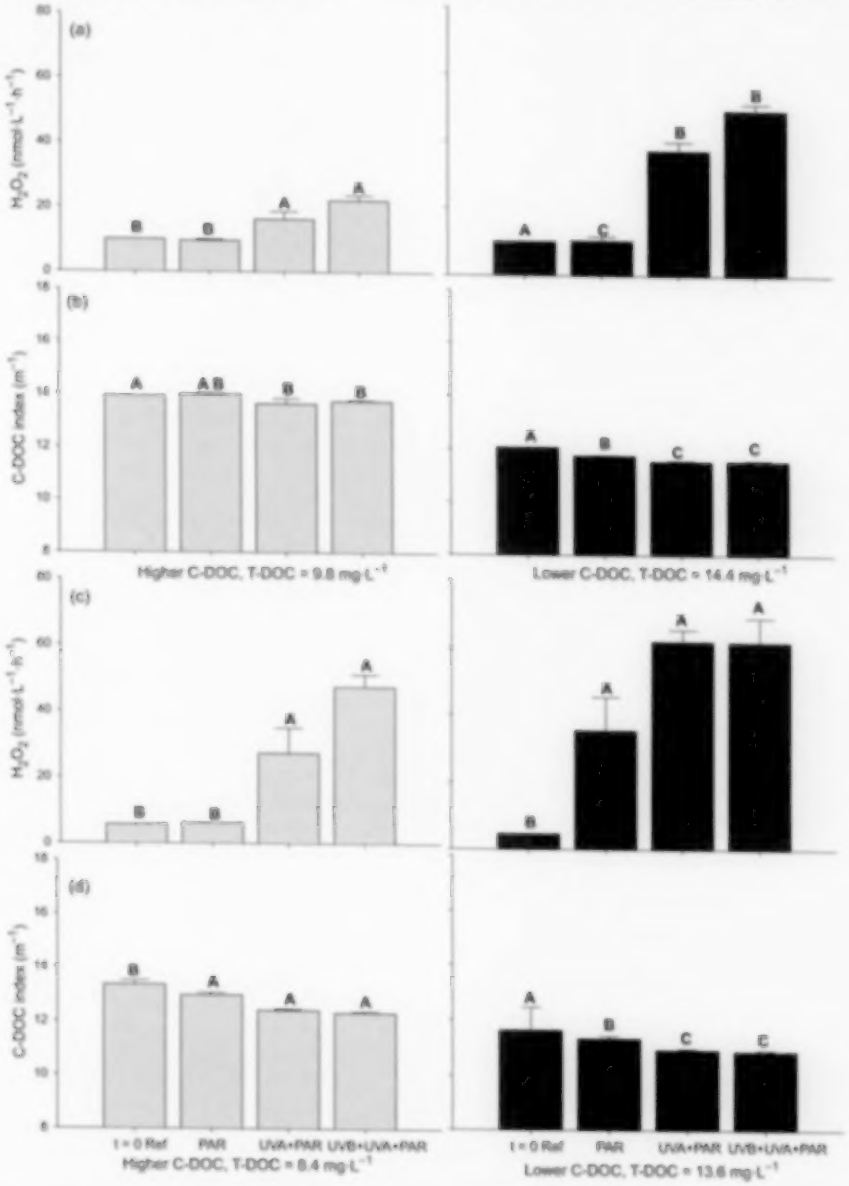
Fig. 7. Cloudy conditions (5 July) results showing (*a*) hourly production of H_2O_2 and (*b*) in situ coloured dissolved organic carbon (C-DOC index expressed as Ka330) in microcosms of lake water from South Lake (solid bars) and Dock Lake (shaded bars). Cloudless conditions (9 July) results showing (*c*) hourly production of H_2O_2 and (*d*) in situ C-DOC (C-DOC index expressed as Ka330) in microcosm of lake water from South Lake and Dock Lake. Microcosms were subject to one of three ultraviolet radiation (UVR) treatments: UVB+UVA is selectively removed (treatment = photosynthetically active radiation (PAR), UVB is selectively removed (treatment = UVA+PAR), and full sunlight (treatment = UVB+UVA+PAR) compared with the initial control at the start of the experiment (treatment = t = 0 Ref). For each analysis, treatments not associated by the same letter are significantly different (α = 0.05).



DOC (e.g., Dock Lake) rather than higher C-DOC (e.g., South Lake), despite the highest rates of photobleaching co-occurring where H_2O_2 build up was strongest. While this result was counter to our expectation, it is consistent with the H_2O_2 pattern relative to C-DOC levels reported by Scully et al. (1997). Furthermore, the highest H_2O_2 buildup did occur where T-DOC was highest in our microcosm and diurnal studies but not in our 40-lake survey. The underlying explanation for the findings of Scully et al. (1996) is that while lakes with higher concentrations of chromophoric DOC have more abundant substrate for photochemical reactions, the more rapid attenuation of solar UVA and UVB wavelengths

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Table 2. One-way analysis of variance (ANOVA) results that tested for differences between lakes in H_2O_2 production and coloured dissolved organic carbon (C-DOC) levels ($\alpha = 0.05$).

	South Lake				Dock Lake			
	$H_2O_2 \cdot h^{-1}$		C-DOC		$H_2O_2 \cdot h^{-1}$		C-DOC	
Factor	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Cloudy	19.5822	0.0005	7.4134	0.0107	159.721	<0.0001	80.5695	<0.0001
Cloudless	49.5631	<0.0001	60.3886	<0.0001	40.6496	<0.0001	183.7544	<0.0001

Note: Each experiment was tested individually (cloudy and cloudless) and differences among all treatments were compared for each lake using Tukey–Kramer's honestly significant difference (HSD).

by C-DOC restrict penetration and attendant photochemical reactions to a relatively shallow zone.

The results, in combination with those of Scully et al. (1997), suggest a second hypothesis that H_2O_2 levels will be highest in lakes with lowest levels of C-DOC. However, when this is tested against the in situ patterns of H_2O_2 levels in a representative set of lakes, this alternative hypothesis is also rejected. In contrast with both alternative hypotheses, H_2O_2 concentrations appear to be highest at mid-C-DOC (equivalently, mid-DOC) levels, in lakes of intermediate flooding frequency. The pattern of peak concentrations in intermediately flooded lakes is evident in all three surveys over the open-water period. We conclude from this that H_2O_2 concentrations do not follow concentrations of either C-DOC or DOC alone. Because lakes in the Delta do not stratify and have fairly similar depths, lake area ought to be related to water column mixing rates, and mean depth is essentially equivalent to mixing depth, but H_2O_2 levels do not appear to be related to either of these parameters (Febria 2005).

A possible difference between our results and that of Scully et al. (1997) is that the lakes studied were all of similar depths (fairly shallow) and showed little variation in mixing depths. One consequence of this is that lower levels of C-DOC may not necessarily result in increased penetration (i.e., statistically significant penetration) of UVR irradiance, which is already to the lake bottom. An additional difference is that lakes with low C-DOC can have relatively high levels of nonchromophoric DOC derived from macrophyte communities (Squires and Lesack 2002; Spears and Lesack 2006). In these particular lakes, there may be optimization processes causing the pattern of peak H_2O_2 levels in lakes with intermediate DOC and intermediate flooding frequencies. Because our results indicate higher H_2O_2 buildup in the lower C-DOC, high T-DOC lake (i.e., Dock Lake) relative to the higher C-DOC lake (i.e., South Lake), it would be useful to repeat this experiment by adding a lake with an intermediate C-DOC level.

There is still much to learn about H_2O_2 dynamics in lakes of the Mackenzie Delta. The use of apparent quantum yields of H_2O_2 (i.e., the number of moles of H_2O_2 formed per mole of photons absorbed at a specific UV wavelength; Cooper and Lean 1989; Scully et al. 1996) or areal H_2O_2 production (i.e., milligrams of H_2O_2 formed on an surface areal basis; Scully et al. 1997) may enhance our understanding of the H_2O_2 patterns and distribution. Daily H_2O_2 production has also been shown to be a function of depth and wavelength (Scully et al. 1997) and DOC concentration (Scully et al. 1996). Regardless, the results of our work have found consistent patterns across a range of lake types and DOC compositions.

In conclusion, this study represents the first investigation that has examined the patterns of H_2O_2 in a system of delta lakes anywhere in the circumpolar Arctic. The results of this study show that H_2O_2 levels are highest in lakes with intermediate levels of C-DOC rather than where C-DOC levels are highest. We also found that H_2O_2 levels are highest around the summer solstice and significantly lower later in the summer and that H_2O_2 levels can progressively accumulate during sequences of cloudless days with 24 h daylight. We initially expected that H_2O_2 buildup would be highest in lake waters where the combination of C-DOC and UVB exposure is highest, but we found that UVA played the most important role in the photobleaching of C-DOC and production of H_2O_2.

Implications of results

For the Mackenzie Delta, global climate models predict that the annual river flooding event will likely occur earlier each spring and peak water levels will likely decline, providing substantially less floodwater to lakes in the Delta (Rouse et al. 1997). Also, lower water levels are expected during the open-water season, with the possibility of some perched lakes drying out completely (Marsh and Lesack 1996). The main consequence of this will be a considerable shift in the DOC regime, with a short-term overall increase in C-DOC into Mackenzie Delta lakes as warming temperatures facilitate melting permafrost. On a global scale, UVB radiation is expected to increase and therefore increase photochemical activity and H_2O_2 production (Zepp et al. 2003). Based on observing major reductions in H_2O_2 levels within a few weeks of the summer solstice, we postulate that the most significant photochemical effects on the seasonal dynamics of aquatic bacteria and other microorganisms are likely to be strong in the first weeks after ice-out and may be relatively weak later during the open-water period. These effects may include increased water clarity earlier in the season, an inhibition of biological processes due to both photochemical reactions and direct UVR, followed by an unknown response by the biological community to the resultant pool of labile, photolyzed DOC substrate that remains for the duration of the open-water season.

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Sperm quality influences male fertilization success in walleye (*Sander vitreus*)

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Abstract: We examined how variation in sperm quality influences a male's success at fertilizing ova (male fertilization success) in a wild population of walleye (*Sander vitreus*). To do this, we conducted controlled fertilization trials using milt and eggs (ova) from wild-spawning fish and measured male fertilization success (percentage of ova fertilized) by examining eggs after 24 h of incubation. We found that both the number of sperm and sperm swimming speed (at 10 s after activation) were significantly related to fertilization success. There was, with respect to fertilization success, a relatively large return on male investment in the number of sperm, but this return diminished as the percentage of ova fertilized increased above 50%. This is in agreement with theoretical predictions based on external fertilization dynamics. When the number of sperm used in the experimental trials was kept constant, variation in sperm swimming speed (at 10 s after activation) explained approximately 90% of the variation in a male's fertilization success. These findings demonstrate that the variation in sperm quality found in wild spawning populations has the potential to dramatically influence male reproductive success.

Résumé : Nous examinons comment les variations de la qualité du sperme influencent le succès des mâles dans la fécondation des oeufs (succès de fécondation mâle) dans une population sauvage de dorés (*Sander vitreus*). Nous avons donc fait des essais contrôlés de fécondation à l'aide de laitance et d'oeufs (ova) de poissons frayant en nature et mesuré le succès de la fécondation (pourcentage d'oeufs fécondés) à l'examen des oeufs après 24 h d'incubation. Il y a une relation significative entre à la fois le nombre et la vitesse de nage des spermatozoïdes (10 s après l'activation) et le succès de la fécondation. Comptabilisé du point de vue du succès de la fécondation, l'investissement des mâles en un nombre élevé de spermatozoïdes est relativement très rentable, mais la rentabilité diminue à mesure que le pourcentage d'oeufs fécondés dépasse 50 %. Cette relation concorde avec les prédictions théoriques basées sur la dynamique de la fécondation externe. Lorsque le nombre de spermatozoïdes est maintenu constant dans les essais expérimentaux, la variation de la vitesse de nage des spermatozoïdes (10 s après l'activation) explique environ 90 % de la variation du succès de la fécondation chez les mâles. Ces résultats démontrent que la variation de la qualité du sperme observée chez les populations sauvages durant la fraye peuvent influencer de façon sérieuse le succès de la fécondation chez les mâles.

[Traduit par la Rédaction]

Introduction

A male fish's fertilization success (percentage of ova fertilized during a spawning event) under sperm competition is thought to be related to both the number of sperm released and the ability of those sperm to swim to and fertilize the ova (Ball and Parker 1996; Stockley et al. 1997). Even when a male spawns without competition from other males, sperm traits would be expected to influence fertilization success and thus overall male reproductive success. Surprisingly, however, there has been relatively little research examining how different sperm traits relate to fertilizing ability (Gage et al. 2004; Malo et al. 2005).

A male's fertilization success is predicted to increase with the number of sperm released by a male during a spawning bout, but the benefit of releasing more sperm should decrease as the total number of sperm approaches the number required to fertilize all of the available ova (Ball and Parker 1996). This relationship has been empirically examined in several externally fertilizing fishes with some support for the model. For example, the male fertilization success of bucktooth parrotfish (*Sparisoma radians*) in natural spawning events increased dramatically with the number of sperm released, reaching an asymptote close to 95% fertilization success (Marconato and Shapiro 1996). The relationship between sperm number and male fertilization success has

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also been examined using in vitro fertilizations with rainbow trout (*Oncorhynchus mykiss*) and sockeye salmon (*Oncorhynchus nerka*) (Hoysak and Liley 2001; Liley et al. 2002), but in these studies, there was much variation in the results, so a precise description of the relationship between male fertilization success and sperm number was not possible.

Male fertilization success should also increase with sperm longevity and swimming speed. The rationale for this is twofold: based on a simple physical model of random sperm movement, it is reasonable to assume that (*i*) the longer period over which sperm swim, the greater the chance of encountering an unfertilized ovum and (*ii*) faster swimming sperm encounter more ova per unit time. The influence of sperm swimming speed on a male's fertilization success is obvious when a male's sperm is competing with those of another male, but it is also important when spawning takes place in turbulent waters and sperm may remain in the vicinity of unfertilized ova for only a few seconds. Even over the 2–3 min that the spermatozoa of many fish species remain motile (e.g., Burness et al. 2004), they would swim <2 cm, at most, and thus must be deposited and remain very close to an unfertilized ovum to have any chance of fertilization.

Despite the theoretical importance of sperm swimming velocity on male fertilization success, there has been relatively little research examining this relationship. A few studies have shown that male fertilization success is positively related to sperm velocity in externally fertilizing marine invertebrates (Au et al. 2002; Kupriyanova and Havenhand 2002) and internally fertilizing Iberian red deer (*Cervus elaphus hispanicus*) (Malo et al. 2005). However, most of the evidence supporting the effect of sperm motility on male fertilization success in fish comes from measuring the proportion of forward-moving sperm and not from actual measurement of sperm swimming velocity (Geffen and Evans 2000). To our knowledge, the only study to examine the relationship between sperm velocity and male fertilization success in a fish species under somewhat natural conditions was conducted by Gage et al. (2004), who showed that the sperm velocity of Atlantic salmon (*Salmo salar*) males was the best predictor of a male's fertilization success under sperm competition. Lahnsteiner et al. (1998) also examined the influence of sperm velocity on in vitro male fertilization success in rainbow trout, but they used a specialized fertilization solution to activate the sperm, and this may not have accurately mimicked natural spawning conditions.

Longer sperm tails are expected to generate more power and enable faster swimming, thus it is predicted that male fertilization success will also increase with sperm tail length (Gomendio and Roldan 1991). Recent evidence suggests that sperm with longer tails do indeed swim faster in bluegill (*Lepomis macrochirus*) (S. Casselman and R. Montgomerie, unpublished data), and fertilization success increases with sperm tail length in the internally fertilizing snail *Viviparus ater* (Oppliger et al. 2003). The length of the end piece of the sperm tail is also related to fertilization ability in Atlantic salmon (Vladić et al. 2002). In that study, it was hypothesized that the end piece may affect the resistance of the sperm tail to viscous drag, such that sperm with longer end pieces experience reduced drag and increased fertilization success.

In this study, we investigated the effects of sperm number as well as sperm swimming speed, longevity, and morphology on male fertilization success in walleye (*Sander vitreus*). We designed our experiments to mimic some of the conditions encountered during natural spawning events — temperature, duration of sperm–ovum contact, and fertilization medium — so that our results could be used to predict the impact of ejaculate quality on reproductive success in wild populations. Walleye spawn in the spring, shortly after ice breaks up, at water temperatures 6–9 °C. Spawning takes place in rocky areas in fast-flowing water at the base of impassable falls or dams in rivers and on coarse gravel shoals in some lakes (Scott and Crossman 1973). Males congregate at the spawning grounds prior to the arrival of females, but males build no nest and are not territorial. At night, one to two females move together into shallow water with up to six males, who compete for fertilizations. Within the spawning group, males will become aggressive, with pursuit, pushing, and fin erection occurring prior to spawning (Scott and Crossman 1973). Both eggs and sperm are broadcast into the water column, where fertilization occurs; eggs then settle into crevices in the substrate where they incubate for 12–18 days before hatching.

Materials and methods

We collected mature walleye from the Bay of Quinte (44°N, 77°W), Ontario, Canada, during spawning in April 2003, using a trap net placed near the spawning grounds where fish were staging. Fish were removed from the net and held in large (200 L), insulated containers (with fresh water regularly renewed) for up to 6 h before being processed.

Fertilization experiments

All fertilizations were conducted in 500 mL clear plastic containers. For each trial, approximately 100 ova and a milt sample were placed about 5 cm apart in the bottom of a dry container. Then, 400 mL of fresh lake water was poured quickly into the container to ensure thorough and immediate mixing of ova and sperm. This also ensured that all sperm and ova came into contact with water at the same time and that all sperm were activated simultaneously. After 30 s, the water was poured off the eggs, and fresh lake water was immediately added to wash off any remaining sperm and thus standardize the amount of time available for fertilization. This final washing was performed so that we could look specifically at the effects of variation in sperm movement and morphology during the period when walleye sperm in nature are most likely to be in the vicinity of unfertilized ova.

After the fertilization trials, eggs were transported to the lab and incubated at 10–12 °C for 24 h. Then, 50 eggs from each trial were examined at 3× magnification to determine fertilization success. Only clear, undamaged eggs were counted, and eggs were categorized as being fertilized if there was segmentation of the blastodisc (Hardy 1978). We define a male's fertilization success as the percentage of ova fertilized during each trial (spawning event).

Sperm number and male fertilization success

To determine the effect of sperm numbers on male fertilization success, we first conducted fertilization trials for 10

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male–female pairs, with six replicates for each pair using different numbers of sperm in each replicate. Ten different male–female pairs were used in this experiment to provide a large sample of random male × female combinations. Each of these fertilization trials was performed immediately after ova and sperm were collected from the live adults, within 10 min of stripping the adults. For each male, we loaded a microhaematocrit (75 mm long; 1.1–1.2 mm inside diameter) capillary tube 70% full with milt and centrifuged it for 2 min at 8000 r·min^{-1} (1 r = 2π rad; 5900*g*). We then calculated spermatocrit as the ratio of packed sperm to total volume of milt (Bouck and Jacobson 1976) so that we could control for differences in sperm density among males. Using a haemocytometer, we determined the number of spermatozoa per microlitre of milt for one male, accurate to within ±5%, then used the spermatocrit for that milt sample as a standard for calculating the number of sperm per microlitre of milt for the other males from their spermatocrit values. Based on a pilot study that we had previously conducted on the fertilization dynamics of walleye, we chose six different densities of sperm to use in each trial to yield male fertilization rates ranging from 100% to 0% of ova fertilized. The volume of sperm added during these fertilization trials was calculated to produce final sperm densities of 500 000, 250 000, 100 000, 50 000, 5000, and 500 sperm·mL^{-1} water in the total volume of 400 mL of lake water used per trial. All six replicates for each male × female pair were performed virtually simultaneously to eliminate any potential order effect due to storage of milt or ova.

Sperm movement, morphology, and male fertilization success

On a different day, we conducted further fertilization trials to determine the influence of sperm traits (swimming speed, tail length) on the fertilization success of 20 different males. To minimize any effect of female identity on male fertilization success, we pooled 50 g of ova (approximately 15 000 ova) from each of five females and used this pooled batch of about 75 000 ova for all fertilizations. We used this pooled sample rather than ova from different females to minimize the effects of differential fertilization success of particular male × female combinations that might influence our results. Thus, each male was tested against the same pooled set of ova. We stored these unfertilized ova in a cooler at 4 °C after collection until we used them in a fertilization trial.

We stripped milt from each of the males immediately prior to conducting the fertilization trials to eliminate any potential change in sperm quality caused by storage. We controlled for differences in sperm density among males by measuring the spermatocrit (as above) of each milt sample used. We then calculated the appropriate volume of milt needed to ensure that the same number of sperm (5×10^4 sperm·mL^{-1}; see Results) was used from each male in the fertilization trials.

Fertilization trials were replicated five times for each male sampled and conducted in dyads (two males at a time) over a period of 8 h, using the protocol described above. It took 20–60 min to complete the trials for each dyad of males, with 60 min breaks after the fourth and sixth dyads, resulting in three groups of dyads. Because the ova were collected before these fertilization trials began, they were stored for different periods, up to 8 h, before being used in a trial.

Sperm motility measurements

We measured sperm motility for each male used in the fertilization experiments within 1 h of sperm collection. A two-step dilution process was used to ensure simultaneous activation of the sperm (Billard and Cosson 1992). To dilute the milt without activating the sperm, we added 1 µL of milt to 750 µL of an extender developed for walleye (Moore 1987), then placed 1 µL of this solution into a haemocytometer chamber. Sperm were then activated by flooding the chamber with lake water (10 °C) taken from the spawning site. To ensure that sperm motility was measured at the same temperature for all samples, the haemocytometer was maintained at 10 °C using a Peltier plate controlled with a variable DC power supply and monitored with a thermocouple. Sperm motility was recorded on videotape using a CCD video camera (Sony model XC-ST50, Hamilton–Thorne Research, Beverly, Massachusetts, USA) mounted on a negative phase contrast Olympus CH30 microscope (Hamilton–Thorne Research) at 100× magnification.

We analyzed the videotapes using a CEROS video sperm analysis system (v. 12, Hamilton-Thorne Research). The smoothed average sperm swimming velocity (VAP, µm·s^{-1}) was measured for a minimum of 25 sperm from each male at 10, 20, and 30 s after water was added to the milt sample (thereby activating the sperm). VAP is calculated using a smoothing algorithm that reduces the effect of lateral head displacement and produces a path representative of the sperm position along the sperm's track of forward movement. The length of this track is then divided by the time elapsed (0.5 s) to determine VAP. To assess the straightness of the swimming path, we also calculated a linearity index (STR) by dividing the straight-line distance (distance between the beginning and the end of the track) traveled in 0.5 s by the total distance traveled in the same period. Percent motility was measured as the proportion of sperm with both VAP > 20 µm·s^{-1} and STR > 0.20 at 10, 20, and 30 s after activation. The VAP criterion is commonly used to distinguish progressively motile fish sperm (e.g., Lahnsteiner et al. 1998), whereas the STR criterion eliminates a few sperm that are not moving forward; sperm not meeting these criteria were judged to be nonmotile.

Sperm morphology measurements

We preserved sperm for morphological analyses by diluting 5 µL of milt in 750 µL of 2% formaldehyde solution. A small drop of this solution was spread thinly across a microscope slide and air-dried. We digitized 10 haphazardly chosen sperm from each male using a microscope at 600× magnification and a CCD video camera. Using NIH Image (Version 1.62, available from http://rsb.info.nih.gov/nih-image/), we measured the tail length of each sperm to the nearest 0.1 µm on the digitized images.

Statistical analyses

We used JMP 5.1 (SAS Institute Inc., Cary, North Carolina) for all analyses. We used repeated measures analyses of variance where appropriate when the sperm from each male was used in different fertilization trials during a single experiment and when analyzing sperm swimming speed at different periods after activation. For models with random effects (i.e., male as factor), we used the restricted maximum

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Fig. 1. Percentage of ova fertilized in relation to experimentally manipulated sperm density in walleye (*Sander vitreus*). Each data point represents an experimental trial with a single male, with sperm from the same 10 males tested at each density. Overlapping data points are jittered so that all data points are shown, except for results at 500 sperm·mL^{-1}, which are shown as triangles with sample sizes indicated for overlapping data points.

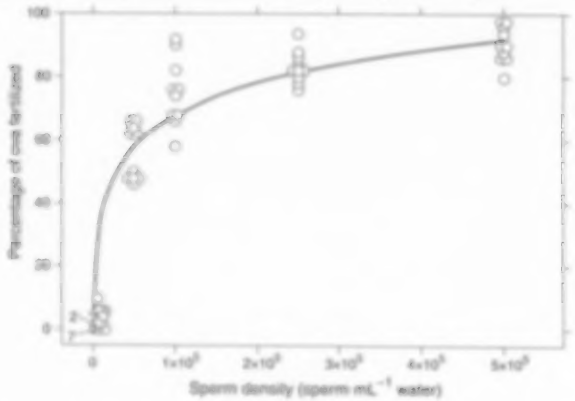
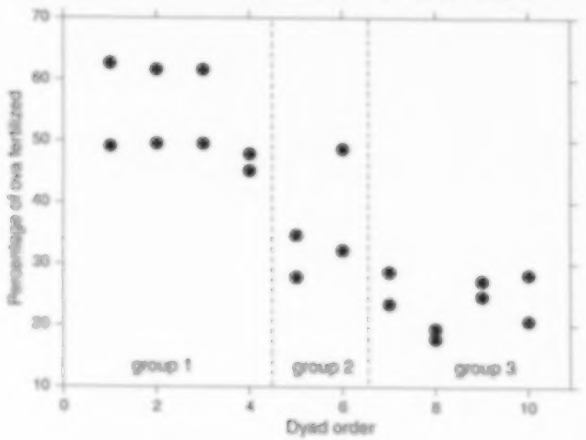


Fig. 2. Decline in mean percentage of ova fertilized with order of fertilization trials. Each data point is the mean of five trials per male, and males were tested simultaneously in dyads. Fertilization groups were delimited, as shown, for further analysis.



likelihood method for analysis. None of the analyses of variance had residuals that departed significantly from normality (Shapiro–Wilk tests, $p > 0.05$), so no transformations were needed. For analyses of covariance, interaction terms were removed if not significant (NS), indicating no significant difference in slopes among treatments.

Results

Sperm number and male fertilization success

As expected, mean male fertilization success varied significantly among trials depending upon the number of sperm used, from 1% (range 0%–6%) with a sperm density of 500 sperm·mL^{-1} water to 90.6% (range 80%–98%) with 5×10^5 sperm·mL^{-1} water (repeated measures analysis of variance, $F_{[9,45]} = 402$, $p < 0.0001$). A logarithmic equation (fertilization success = $33.8 \times \log_{10}$(sperm density) – 101.7) provided a good description of the relationship between fertilization success and the number of sperm ($r^2 = 0.89$, $p < 0.0001$; Fig. 1).

Based on this analysis, we used a density of 5×10^4 sperm·mL^{-1} water to examine the effect of sperm quality on male fertilization success in subsequent experiments. The fertilization success predicted for this sperm density (58%) was intermediate, with no replicates having close to 0% or 100% fertilization success (Fig. 1) and thus little danger of truncated results that might complicate the analysis.

Sperm movement, morphology, and male fertilization success

The order in which the fertilizations were performed had a significant effect on mean male fertilization success (Spearman's rank correlation, $r = -0.87$, $p < 0.0001$, $n = 20$ males in 10 dyads), in that mean male fertilization success decreased over the course of the whole experiment (Fig. 2). This decline in male fertilization success was not due to changes in sperm quality, as males were stripped of milt immediately prior to each dyad of trials, and thus the time between stripping milt and sperm activation was the same for each male. Moreover, sperm swimming speed (VAP at 10 s after activation) increased rather than decreased over the course of the experiment ($r = 0.48$, $p = 0.03$, $n = 20$ males in 10 dyads) for no obvious reason. We believe that this decline in male fertilization rates over the course of the experiment was likely due to reduced ovum fertility resulting from storage, possibly because of decreased oxygen supply or ovum hardening caused by accidental exposure to moisture (condensation). For further analysis, we included either dyad order or group (see Methods) in the models to control for order effects.

VAP at 10 s after activation was significantly and positively related to male fertilization success (repeated measures analysis of covariance, $F_{[1,80]} = 8.9$, $p = 0.004$, interaction term NS and removed from the model; Fig. 3), with dyad as covariate to control for order effects. In this model, VAP explained 72% of the variation in fertilization success, and the slope of the relationship between percent fertilization and VAP was 0.70. Using group as a covariate instead of dyad also revealed a significant relationship between fertilization success and VAP at 10 s after activation (repeated measures analysis of covariance, $F_{[1,80]} = 16.2$, $p = 0.0001$, interaction term NS and removed from the model), with a slope of 0.75 for the relationship between percent fertilization and VAP. Within all three groups, male fertilization success increased with VAP at 10 s after activation (Fig. 3), but this relationship was only significant for the first two groups of trials ($p \leq 0.05$, repeated measures analyses of variance within groups), with regression slopes of 0.80 (group 1) and 1.04 (group 2).

Male fertilization success was not related to VAP at 20 and 30 s after activation (repeated measures analyses of covariance, both $F_{[1,79]} < 2.57$, both $p > 0.11$, with group as a factor) nor to STR at 10, 20, and 30 s after activation (repeated measures analyses of covariance, all $F_{[1,79]} < 0.49$, all $p > 0.49$, with group as a factor). Similarly, the proportion of

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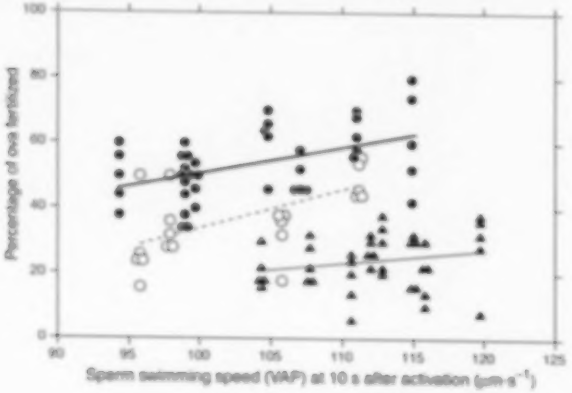
Fig. 3. Increase in fertilization success with average sperm velocity (VAP) at 10 s after activation within fertilization groups as defined in Fig. 2 (group 1 presented in solid circles, group 2 presented in open circles, and group 3 presented in solid triangles). Five trials were run with the sperm from each male; overlapping data points are jittered so that all data points are shown; regression lines from repeated measures analyses are shown.



motile sperm at 10, 20, and 30 s after activation did not influence male fertilization success (repeated measures analyses of covariance, all $F_{[1,70]} < 2.22$, all $p > 0.14$, with group as a factor).

Mean sperm tail length varied significantly among males (analysis of variance, $F_{[19,180]} = 4.62$, $p < 0.0001$, $n = 10$ sperm·male^{-1}). Despite this variation, there was no effect of sperm tail length on fertilization success (repeated measures analysis of covariance, $F_{[1,16]} = 0.27$, $p = 0.61$, with group as a factor). Nor was mean sperm swimming velocity (VAP) at any time after activation related to mean sperm tail length across males (at 10 s: $r = 0.16$, $p = 0.50$; at 20 s: $r = 0.30$, $p = 0.19$; at 30 s: $r = 0.03$, $p = 0.90$; $n = 20$ males).

Discussion

Our experiments demonstrated a significant effect of sperm number on male fertilization success as expected. At the highest sperm density (5×10^5 sperm·mL^{-1} water), male fertilization success averaged 91%. Although this is less than compete fertilization success, it is in agreement with previous studies that have found maximum male fertilization success of walleye under hatchery conditions to be 75%–86% (Moore 1987, 2003), likely due to variability in ovum quality (Moore 2003). In contrast, average male fertilization success was only 1% with sperm density of 500 sperm·mL^{-1} water.

While previous studies have found male fertilization success to be highly variable, ranging from 8% to 97% under constant experimental conditions (Hoysak and Liley 2001), we found much less variation in male fertilization success when the density of sperm was held constant. The variation in fertilization success found in other studies may have been caused by embryo mortality during incubation, as eggs were often incubated 1–3 weeks before measuring fertilization success, and mortality is often quite high during this period (S. Casselman, personal observation). In Hoysak and Liley (2001), for example, cloudy eggs were simply removed from the sample during the incubation period, apparently without assessing whether or not they contained an embryo. To minimize this potential source of variation, we incubated the eggs ≤24 h before assessing the fertilization status of the sample.

The shape of the relationship between male fertilization success and sperm number suggests that there is initially a relatively large return on male investment in ejaculates but that this return diminishes as the percentage of ova fertilized increases above 50%. For example, doubling the density of sperm from 1×10^5 to 2×10^5 sperm·mL^{-1} water increased predicted male fertilization success from 67% to only 77%. Thus, in the absence of sperm competition, the percentage of ova in a clutch that is fertilized by an individual male is directly related to the number of sperm released, and thus males should adjust ejaculate size depending upon both their prospects for further spawnings and their rate of sperm production (Shapiro and Giraldeau 1996). However, when a male competes with other males during a spawning event, other factors like sperm swimming speed and sperm–ovum interactions can influence a male's success (Gage et al. 2004).

For example, when the density of sperm in water was held constant in our fertilization trials, sperm swimming speed at 10 s after activation was significantly related to male fertilization success. Thus, in our first group, males with the fastest-swimming sperm had approximately 40% greater fertilization success than males with the slowest-swimming sperm. Across all trials, the slope of the relationship between percent fertilization success and VAP was 0.70–0.75; within the first two trial groups, this was even higher at 0.80–1.04. Thus for every increase of 1 μm·s^{-1} in sperm swimming speed (VAP), a male's fertilization success would increase by about 0.75%. Over the range of swimming speeds (VAP at 10 s after activation) observed in this study (94.3–119.7 μm·s^{-1}), this translates into an increase of 18.8% in male fertilization success. Thus, we conclude that the variation in sperm swimming speeds that we observed can have a major impact on male reproductive success.

Although our fertilization experiments were conducted using sperm from each male in isolation, our results can be extended to make predictions regarding the outcome of sperm competition involving males with ejaculates of different quality. Thus, if multiple males release the same number of sperm, the male with the fastest swimming sperm should fertilize the greatest percentage of ova and that percentage may depend upon the density of the sperm in the water, the distribution of unfertilized ova, the timing of ejaculation, and the distribution of sperm swimming speeds (R. Montgomerie and S. Casselman, unpublished data). In an experimental study of the effects of sperm traits on male fertilization success in the Atlantic salmon, Gage et al. (2004) found that sperm swimming velocity was the best predictor of male fertilization success when milt from different males competed for access to ova, even when the relative sperm densities of two different males were in a 2:1 ratio. Thus, in that study, the effect of relative sperm density was not significant, presumably swamped by the variation in sperm swimming speed. Although Gage et al. (2004) did attempt to mimic natural spawning conditions in their experiments,

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they kept mean sperm density constant so that they could examine the effects of relative swimming speeds and numbers of sperm. In nature, however, the density of sperm in water in the vicinity of unfertilized ova during spawning is likely to vary considerably because of varying turbulence in the water, distances between the sexes when gametes are released, and the relative timing of gamete release by the male(s) and female. In the present study, we showed that both factors are likely to be important determinants of male fertilization success, but more work will be needed to determine the relative importance of sperm swimming speeds and ejaculate size at sperm:ovum densities found in nature.

While there was an effect of sperm swimming velocity on male fertilization success in walleye when measured at 10 s after activation, we found no such relationship at 20 or 30 s after activation. The swimming speed of walleye sperm declines rapidly after activation, such that between 10 and 30 s after activation, swimming speed declines by about 70% (S. Casselman and R. Montgomerie, unpublished data). Previous work on the fertilization dynamics of salmon and rainbow trout found that the majority of fertilization took place within the first 20 s after activation (Hoysak and Liley 2001; Liley et al. 2002), so that speed measurements after this period are expected to be irrelevant to a male's fertilization success.

Interestingly, there was no effect of the differences among males in percent sperm motility on male fertilization success. Assuming that nonmotile sperm are incapable of fertilization (Levanduski and Cloud 1988), any decline in the proportion of motile sperm should result in decreased male fertilization success. In support of this, a positive relationship has been found between percent motility and male fertilization success in rainbow trout (Geffen and Evans 2000), but not in the serpulid polychaete *Galeolaria caespitosa* (Kupriyanova and Havenhand 2002). One possible explanation for our results is that percent motility may only be expected to affect male fertilization success when all other sperm traits are equal and when differences in percent motility dramatically influence sperm density in the water during spawning. Moreover, when the ratio of sperm to ova is very high, the fastest-swimming sperm might be expected to fertilize the majority of ova even when a high proportion of sperm are immotile, as long as the density of motile sperm is high. In our study, mean sperm motility at 10 s after activation was 79.8% (±6.4%, 95% confidence level, $n = 20$ males) and thus relatively high and not very variable, so an effect of motility may have been absent or merely difficult to detect.

We also found that there was no effect of sperm tail length on male fertilization success. This is initially surprising because theoretical models predict that sperm with longer tails swim faster, and there is some empirical evidence in support for this (Lahnsteiner et al. 1998; Jobling et al. 2003; Burness et al. 2004). Thus, if sperm tail length influences sperm swimming speed, then tail length should be related to fertilization success (Ball and Parker 1996). However, we found no relationship between sperm morphology and sperm swimming speed, so there was no reason to expect an effect of sperm tail length on fertilization success in this species. Similarly, Gage et al. (2002) found no relationship between sperm swimming speed and sperm flagellum length in Atlantic salmon, but again, in that species, sperm swimming speed was a major determinant of a male's fertilization success under (experimental) sperm competition (Gage et al. 2004). Undoubtedly sperm swimming speed is determined by factors other than, or maybe in addition to, sperm length, and this subject deserves more detailed study.

This study demonstrates the importance of sperm number and swimming velocity in determining male fertilization success and suggests that males able to produce large numbers of fast-swimming sperm will benefit from increased reproductive success. Further study is required to determine the impact of percent sperm motility and morphology on fertilization success when controlling other sperm traits. As this experiment was conducted in vitro, the effect of these sperm traits on fertilization success in natural spawning events should also be examined.

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Variation in the response of crustacean zooplankton species richness and composition to the invasive predator *Bythotrephes longimanus*

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Abstract: The predacious invertebrate *Bythotrephes longimanus* has now invaded >90 freshwater lakes in North America. There is some evidence that *B. longimanus* has a negative effect on summer zooplankton species richness; however, no study has examined the effect of *B. longimanus* throughout the ice-free season in more than one lake. We visited 10 invaded and 4 reference lakes every 2 weeks from May to September, collecting *B. longimanus*, crustacean zooplankton, and water chemistry samples. Composite samples were pooled across the study season for each lake. *Bythotrephes longimanus* significantly reduced cladoceran species richness, diversity, and abundance, and the total zooplankton community also exhibited decreased richness, diversity, and abundance. Seasonal sampling was better than synoptic surveys at detecting changes in abundance, but richness estimates were similar. As *B. longimanus* continues to spread across lake landscapes, we expect it will have profound impacts on local and regional richness and species distribution patterns.

Résumé : L'invertébré prédateur *Bythotrephes longimanus* a maintenant envahi >90 lacs d'eau douce en Amérique du Nord. Il y a des indications que *B. longimanus* a un effet négatif sur la richesse spécifique du zooplancton d'été; cependant, aucune étude n'a examiné les effets de *B. longimanus* au cours de la période sans glace dans plus d'un seul lac à la fois. Nous avons visité 10 lacs envahis par le prédateur et 4 lacs témoins à toutes les 2 semaines de mai à septembre et prélevé des *B. longimanus*, des crustacés zooplanctoniques et des échantillons d'eau pour analyse chimique. Nous avons amalgamé les échantillons de toute la période pour chacun des lacs. *Bythotrephes longimanus* réduit significativement la richesse spécifique, la diversité et l'abondance des cladocères; la communauté totale du zooplancton subit aussi une réduction de richesse, de diversité et d'abondance. L'échantillonnage saisonnier permet mieux que les inventaires synoptiques de détecter les changements d'abondance, mais les estimations de richesse sont semblables par les deux méthodes. À mesure que *B. longimanus* continue de se répandre dans les paysages lacustres, nous prévoyons qu'il aura des répercussions importantes sur les patrons de richesse et de répartition d'espèces aux échelles locale et régionale.

[Traduit par la Rédaction]

Introduction

Bythotrephes longimanus Leydig (Crustacea, Onychopoda) is a predatory zooplankton species that has invaded numerous lakes in North America. First detected in Lake Ontario in 1982 (Mills et al. 2003), *B. longimanus* has spread to all of the Laurentian Great Lakes and >90 inland lakes in Ontario (C. Gamble, Ontario Federation of Anglers and Hunters, 4601 Guthrie Dr., Peterborough, ON K9J 8L5, Canada, unpublished data), Minnesota (M. Brown, Department of Biology, University of Minnesota–Duluth, Duluth, MN 55812, USA, unpublished data), and Michigan (Jarnagin et al. 2000) and reservoirs in Ohio (Ferry and Wright 2002). *Bythotrephes longimanus* occupies lakes that span a gradient of temperature, salinity, pH, and depth within its native range in Eurasia (Grigorovich et al. 1998). Humans are an important dispersal vector for *B. longimanus*, and with its broad tolerance of environmental conditions, it appears to have the potential to invade a large number of lakes in North America (MacIsaac et al. 2000, 2004).

Our understanding of the impacts of *B. longimanus* on inland aquatic communities in North America is incomplete. Invasion events are unpredictable, thus little empirical data documenting the effects of invasion on recipient communities exists. However, a single case study of long-term biweekly records of both pre- and post-invasion planktonic communities from Harp Lake, Ontario, has revealed evidence of negative effects of *B. longimanus* on crustacean

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zooplankton species richness and cladoceran zooplankton abundance since its invasion in 1993 (Yan et al. 2001, 2002).

Although trends seen in Harp Lake suggest that the invasion of *B. longimanus* will have important consequences for zooplankton communities, effects of predation can be site-specific (Posey and Hines 1991). Broader spatial surveys are necessary to determine how universal the impacts of *B. longimanus* are in temperate lakes. A study comparing 17 invaded boreal shield lakes to 13 uninvaded reference lakes provided strong evidence that the patterns of invasion observed in Harp Lake were also expressed in other lakes of differing water chemistry, morphometry, and species assemblages (Boudreau and Yan 2003). However, sampling for this study was limited to a single midsummer vertical zooplankton haul, which can underestimate the annual species pool by 50% (Arnott et al. 1998). As well, *B. longimanus* abundances generally peak in midsummer in boreal lakes (J. Young and E. Parrott, Department of Biology, York University, Toronto, ON M3J 1P3, Canada, unpublished data); however, they tend to be absent from the pelagic zone in the early and latter months of the ice-free season. If some zooplankton species are able to take advantage of vernal and autumnal temporal refuges that exist when *B. longimanus* are rare or absent from the water column, then Boudreau and Yan (2003) may have overestimated the effect of *B. longimanus* on annual zooplankton species pools. For example, some copepods may change the timing of reproduction in order to reduce overlap with predators that peak later in the summer (Hairston, Jr., and Walton 1986), and *Daphnia* clones have exhibited earlier age at maturation in the presence of fish kairomones (Sakwińska 2002).

The primary objective of this study was to examine the effects of *B. longimanus* on ice-free season averages of species richness, diversity, evenness, and abundance of crustacean zooplankton in boreal shield lakes. We expected that *B. longimanus* would cause declines in all these indices of community structure. However, because of the inclusion of time periods when *B. longimanus* was absent from the water column, there may be temporal refuges available for some species, during which time they might prosper. We used the persistence of *B. longimanus* in the water column of our study lakes as a way to assess the availability of a temporal refuge for zooplankton. Therefore, high persistence would suggest that there is a short time available for zooplankton to avoid *B. longimanus* and we would expect to see greater negative effects compared with times of low persistence of the invader, when zooplankton would have an opportunity to survive in the absence of predation by this invertebrate. Hence, our seasonal purview might actually detect reduced effects in comparison with those observed during peak *B. longimanus* abundance (Boudreau and Yan 2003; Barbiero and Tuchman 2004), or indeed no effects at all.

A secondary objective of this study was to assess the relationship between species richness and several environmental factors that are typically related to the number of crustacean zooplankton species in a lake. In general, species richness is positively related to lake area (Dodson 1992) and has a hump-shaped relationship with lake productivity (Dodson et al. 2000). In addition, invertebrate predators have been shown to be important in structuring zooplankton communities. In a survey of 60 lakes on the Precambrian Shield, Keller and Conlon (1994) found that a number of zooplankton species common to the region, such as *Daphnia galeata mendotae* and *Diaphanosoma birgei*, were completely absent from fishless lakes containing the phantom midge larvae, *Chaoborus americanus*, a factor attributed to invertebrate predation. Although our lakes were not chosen to span large gradients of area or productivity, we believe that *B. longimanus* may alter these conventional relationships and will be an important driver of ecosystem change.

Materials and methods

Twenty lakes in the districts of Muskoka, Haliburton, and Parry Sound in southcentral Ontario, Canada, were chosen for the survey. The lakes are oligo- to meso-trophic and circumneutral in pH, as is typical of many boreal shield lakes (Table 1). All of the study lakes have forested catchments (mixed deciduous and coniferous) and small littoral zones and are used primarily for recreation. Cisco (*Coregonus artedi*), rainbow smelt (*Osmerus mordax*), lake whitefish (*Coregonus clupeaformis*), and yellow perch (*Perca flavescens*) are the primary planktivorous fish in these lakes (Ontario Ministry of Natural Resources: S. Scholten and E. McIntyre, District of Parry Sound, Parry Sound, ON P2A 1S4, Canada, and S. Sandstrom, District of Muskoka, Bracebridge, ON P1L 1W9, Canada, unpublished data). All the study lakes were chosen to have a similar glacial history, and therefore, there were equal probabilities of including zooplankton species dispersed by glacial retreat. Although our intention was to have equal numbers of invaded and reference lakes, *B. longimanus* was detected in six of our planned reference lakes, reducing their number to four. Hence, we reported results from 14 study lakes, 10 invaded and 4 reference.

Crustacean zooplankton were sampled every 2 weeks from May to September 2003 ($n = 9$) at a deep hole in each lake, located each time using GPS (global positioning system). Sample volume varied slightly among dates because of small differences in station depth (Table 1). A single zooplankton sample was taken by pulling a conical net (mesh size 110 µm, 0.5 m diameter) through the water column, starting 5 m off the lake bottom. Samples were preserved in 5.5% sugared and buffered formalin, and following preservation, a seasonal composite sample was obtained by combining equal proportions from each of the nine biweekly zooplankton samples. Rotifers were sampled from the epilimnion in a concurrent study with an integrated tube sampler (2.5 cm inner diameter) (Hovius et al. 2006). On each sampling date, *B. longimanus* was sampled at five stations in each lake, including the deep hole, with a conical net (mesh size 400 µm, 0.5 m diameter). The stations were <1 km apart. A single haul was taken at each station from 5 m off the lake bottom to the water surface. Mean ice-free *B. longimanus* abundance in the 10 invaded lakes was estimated by averaging *B. longimanus* abundance in the five replicate hauls in each lake over eight sampling dates from June to September 2003. Only eight dates were used, as *B. longimanus* samples were not taken in May. The number of sampling dates when *B. longimanus* was present and its

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Table 1. Physical and chemical characteristics of study lakes.

Lake	Latitude (N), longitude (W)	Z_{max} (m)	Composite sample mean depth (m)	Area (ha)	Calcium ($mg \cdot L^{-1}$)	DOC ($mg \cdot L^{-1}$)	Conductivity ($\mu mhos \cdot cm^{-1}$)	pH	TP ($\mu g \cdot L^{-1}$)
Reference									
Buck	45°25′, 79°23′	23.0	20.6 (1.7)	656.0	2.6	10.7	25.6	6.53	13.30
Doe	45°32′, 79°25′	23.0	12.6 (1.5)	1 187.0	3.6	7.0	46.0	7.08	12.00
Pickerel	45°41′, 79°18′	38.0	26.7 (1.1)	513.0	2.8	6.7	32.4	6.82	8.10
Sand	45°37′, 79°10′	59.0	37.4 (5.3)	568.2	3.5	6.0	37.2	6.78	7.10
Mean		35.8	24.3*	731.1	3.1	7.6	35.3	6.75	10.13*
Invaded									
Kashagawigamog	44°59′, 78°36′	39.7	31.1 (0.8)	817.9	10.8	6.9	95.4	7.56	7.41
Skeleton	45°15′, 79°27′	64.7	53.6 (3.3)	2 155.5	3.8	2.6	44.0	6.94	3.82
Fairy	45°20′, 79°11′	69.5	50.8 (3.6)	711.5	3.5	5.9	49.6	6.95	6.98
Mary	45°15′, 79°15′	56.4	48.9 (1.4)	1 065.4	3.4	6.6	51.0	6.96	7.21
Lake Muskoka	45°03′, 79°28′	66.5	40.4 (0.9)	12 206.0	3.6	5.5	54.4	7.02	5.50
Lake of Bays	45°15′, 79°04′	70.1	39.3 (3.9)	6 904.1	2.9	5.1	36.4	6.97	3.38
Harp	45°23′, 79°07′	37.5	31.2 (1.2)	71.7	3.0	6.7	38.0	6.98	7.22
Vernon	45°20′, 79°17′	37.2	28.8 (3.7)	1 505.1	3.0	7.1	36.8	6.75	7.90
Bernard	45°45′, 79°23′	47.9	40.3 (0.7)	2 057.7	4.1	3.4	65.2	7.13	9.56
Peninsula	45°20′, 79°06′	34.1	21.9 (0.8)	864.8	4.4	6.1	70.6	7.14	9.00
Mean		52.4	38.6*	2 836.0	4.3	5.6	54.1	7.04	6.80*

Note: Standard deviation of mean depth of the composite sample is indicated in parentheses. A *t* test was performed to contrast values in reference and invaded lakes, where an asterisk (*) indicates $p < 0.05$. Lakes are sorted by increasing *B. longimanus* abundance. Z_{max}, maximum depth; DOC, dissolved organic carbon; TP, total phosphorus.

Table 2. Biotic characteristics of study lakes.

Lake	Mean chl *a* ($\mu g \cdot L^{-1}$)	Sample dates *Bythotrephes* present	Persistence of *Bythotrephes*	Lake herring	Rainbow smelt	Lake whitefish	Yellow perch
Reference							
Buck	2.8	0	0	A/P	A	A	P
Doe	3.4	0	0	L	A	L	P
Pickerel	4.4	0	0	L	A/P	A	P
Sand	2.0	0	0	L	H	A	P
Mean	3.1*	0	0				
Invaded							
Kashaga-wigamag	2.0	5	0	M/H	L/M	A/P	P
Skeleton	0.7	6	0	L	L/M	M	P
Fairy	2.2	7	0	A	M	A	P
Mary	2.5	8	0	A	M	A	P
Lake Muskoka	2.9	8	1	VL	N/A	VL	P
Lake of Bays	1.7	7	1	L/M	M	M/H	P
Harp	1.7	7	2	H	A	A	P
Vernon	2.8	7	4	L	M	A/P	P
Bernard	2.8	7	2	A/P	L/M	H	P
Peninsula	2.6	8	6	A	M	A	P
Mean	2.2*	7	1.6				

Note: Persistence is defined as number of consecutive sampling dates that *B. longimanus* abundance was >5 individuals·m^{-3}. Categorical abundance of planktivorous fish provided by Ontario Ministry of Natural Resources (S. Taylor, District of Parry Sound, Parry Sound, ON P2A 1S4, Canada, and S. Sandstrom, District of Muskoka, Bracebridge, ON P1L 1W9, Canada, unpublished data). A *t* test was performed on chlorophyll *a* (chl *a*) concentrations in reference and invaded lakes, where an asterisk (*) indicates $p < 0.05$. Lakes are sorted by increasing *B. longimanus* abundance. A, absent; A/P, absent, but detected in the past; VL, very low; L, low; M, medium; H, high; N/A, not available; P, present.

persistence in invaded lakes are described (Table 2). Persistence was defined as the number of consecutive sampling dates that *B. longimanus* abundance in the lake was >5 individuals·m^{-3}. It has been suggested that there may be an effect threshold of *B. longimanus* at abundances of 3–5 individuals·m^{-3}, especially with regards to their main prey, cladocerans (Boudreau and Yan 2003). The higher threshold value (5 individuals·m^{-3}) was chosen, as it is a more conser-

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vative estimation of *B. longimanus* effects on the zooplankton community.

Zooplankton were enumerated using a protocol designed to ensure reasonable representation of rare species (Girard and Reid 1990). Subsamples were generated with a Folsom plankton splitter, and a mean of 336 individuals (standard deviation = 109) was enumerated. The remainder of the sample was scanned for rare species. Juvenile copepods were excluded from all analyses, except total abundance. Zooplankton were identified and counted on a Leica MZ 12.5 dissecting microscope. Because of uncertainty in identification, *Bosmina* (*Bosmina*) *freyi* and *Bosmina* (*Bosmina*) *liederi* (Taylor et al. 2002) were pooled, as were *Chydorus sphaericus* and *C. brevilabris* (Frey 1980). Species presence or absence and taxonomic revisions are listed in Table S1.[2]

Water chemistry samples were taken the week of 14 July 2003. An integrated tube sampler (2.5 cm inner diameter) was used to collect water from the epilimnion, which was filtered through 80 µm mesh to remove large particles, especially zooplankton. Calcium, dissolved organic carbon (DOC), conductivity, pH, and total phosphorus (TP) were analyzed following Ontario Ministry of the Environment (1983) protocols (Table 1). Chlorophyll *a* (chl *a*) samples were collected every 2 weeks by concentrating a known volume of epilimnetic water onto a 0.7 µm glass fibre filter, extracting for 24 h in methanol, and analyzing with a fluorometer (Table 2) (TD 700; Turner Designs, Sunnyvale, California) (Welschmeyer 1994).

We used two methods to assess the adequacy of our zooplankton richness estimates. First, we compared observed species richness, the number of species found in the composite sample, with Chao's index (S_{Chao1}), a nonparametric measure recommended for estimating species richness, because it does not require knowledge of the species abundance distribution (Magurran 2004). S_{Chao1} was calculated by

$$(1) \qquad S_{Chao1} = S_{obs} + \frac{F_1^2}{2F_2}$$

where S_{obs} is the number of species observed in the sample, F_1 is the number of species in the sample that are represented by a single individual, and F_2 is the number of species that are represented by two individuals (Chao 1984). This estimator will exceed observed species richness as the relative frequency of singletons increases, providing an estimate of total richness based on the chance that some rare species might be missed (Magurran 2004). The second approach to assessing our richness estimates was to determine if our counting effort was sufficient to sample all species present by examining the relationship between number of individuals counted and richness. The difference in species richness using Chao's index was negligible ($t = -0.05$, $p = 0.96$), as was the relationship between number of individuals counted and richness ($r^2 = 0.10$, $p = 0.29$), suggesting that our observed richness is probably representative of the number of crustacean zooplankton species in the pelagic region of the lakes. *Bythotrephes longimanus* was not included in richness estimates.

Species diversity and evenness were assessed using the Shannon–Wiener diversity index and Smith and Wilson's (1996) evenness index, E_{var}, respectively. The values of E_{var} fall between 0 (minimum evenness) and 1 (maximum evenness),

$$(2) \qquad E_{var} = \left(\frac{2}{\pi \arctan\left\{ \sum_{i=1}^{S} \left(\ln n_i - \sum_{j=1}^{S} (\ln n_j)/S \right)^2 \Big/ S \right\}} \right)$$

where n_i is the number of individuals of species i, n_j is the number of individuals of species j, and S is the total number of species. E_{var} is recommended as the best evenness measure for general use (Smith and Wilson 1996).

Multivariate ordination techniques were used to evaluate species abundances in relation to environmental variables and the presence of *B. longimanus*. A preliminary examination using correspondence analysis suggested that linear ordination analysis was appropriate, thus redundancy analysis (RDA) was performed using Canoco 4.5 (ter Braak and Šmilauer 2002). *Bythotrephes longimanus* was entered as a categorical variable to maximize differences between the levels of the treatments. Environmental variables were assessed for collinearity if the variance inflation factor was >10 (Quinn and Keough 2002). All of the significant predictors were independent. All species represented by adults were included in the analysis, and abundances were log-transformed to reduce the influence of dominant species. Forward selection identified the variables that explained the most variance in species data, which were then tested for significance using a Monte Carlo permutation test (499 permutations).

Multiple regression analyses were performed to determine what proportion of the variation in species richness data could be explained by *B. longimanus* abundance and persistence, presence or absence of dominant planktivorous fish species, and environmental variables (i.e., TP, pH, maximum depth, and lake area), using the best subsets procedure (StatSoft Inc. 2001). Planktivorous fish data were provided from sampling by the Ontario Ministry of Natural Resources, but because of uncertainty in quantifying fish abundance, the major planktivores were placed in categories of abundance (Table 2). For the multiple regression analysis, abundances were converted to categorical numerical values (absent = 0, absent but detected in the past = 0, very low = 1, low = 2, medium = 3, high = 4) and averaged for each lake to create an index of fish density. Yellow perch could only be classed as present or absent, and as they were present in all lakes, they were excluded from analyses. Collinearity of environmental predictor variables was detected if the variance inflation factor of any predictors was >10 (Quinn and Keough 2002). Chl *a* was removed from the analysis because of

[2] Supplementary data for this article are available on the journal Web site (http://cjfas.nrc.ca) or may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Building M-55, 1200 Montreal Road, Ottawa, ON K1A 0R6, Canada. DUD 5070. For more information on obtaining material refer to http://cisti-icist.nrc-cnrc.gc.ca/irm/unpub_e.shtml.

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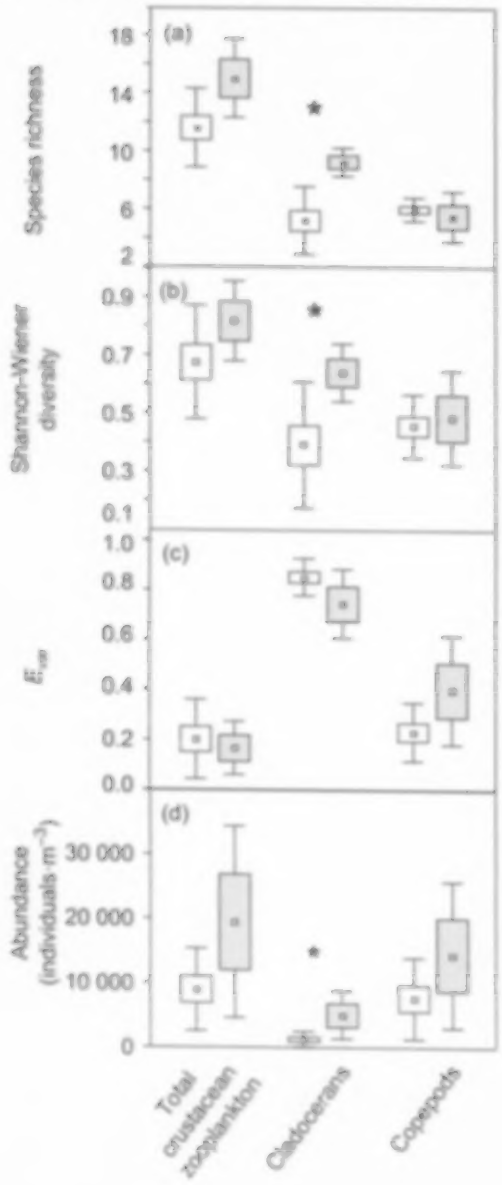
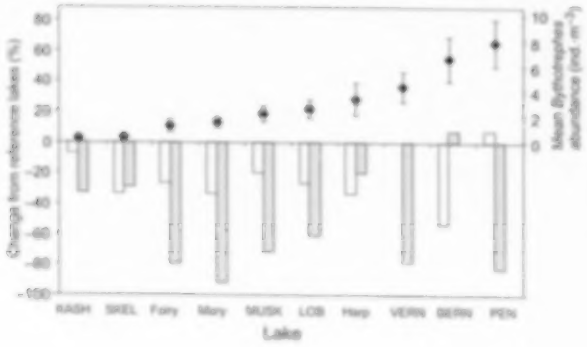
Fig. 1. Box-and-whisker plots of (*a*) species richness, (*b*) Shannon–Wiener diversity, (*c*) E_{var}, and (*d*) abundance (individuals·m^{-3}) for total crustacean zooplankton, cladocerans, and copepods in invaded (open boxes; $n = 10$) and reference (shaded boxes; $n = 4$) lakes. The centre is the mean, the box is the standard error, the whiskers are the standard deviation, and the asterisk (*) is $p < 0.05$.



collinearity with TP. Assumptions of normality, heterogeneity of variance, and independence were also assessed. The best models were chosen by using several criteria: adjusted r^2, Mallow's C_p, and Akaike's information criterion (AIC) (Quinn and Keough 2002). When evaluating the models, smaller values of Mallow's C_p and AIC indicate the best fit.

Fig. 2. On the left axis, percent change in invaded lakes richness (open bars) and abundance (shaded bars) from the average of the reference lakes, contrasted with mean *B. longimanus* abundance (◆; individuals (ind.)·m^{-3}) on the right axis. Vertical error bars represent ± standard error. Abbreviated lake names: BERN, Bernard; KASH, Kashagawigamog; LOB, Lake of Bays; MUSK, Lake Muskoka; PEN, Peninsula; SKEL, Skeleton; VERN, Vernon.



Results

Physical and chemical characteristics did not differ between invaded and uninvaded lakes, with the exception of total phosphorus and chl *a*, which were, on average, 3.33 µg·L^{-1} and 0.94 µg·L^{-1} greater in the uninvaded lakes, respectively (TP, $t = -2.45$, $p = 0.03$; chl *a*, $t = -4.83$, $p < 0.01$) (Tables 1, 2). There were no significant differences between invaded and uninvaded lake calcium (log(x + 1)-transformed), DOC, conductivity, and pH (Table 1). There was also no difference in general lake morphometry, as both maximum depth (Z_{max}) and surface area (log(x + 1)-transformed) were similar between lake categories (Table 1).

In comparisons of invaded and noninvaded lakes, we found that there was a large reduction in observed species richness of zooplankton taxa in invaded lakes, from an average of 15.0 in uninvaded lakes down to 11.6 species in invaded lakes ($t = -2.12$, $p = 0.06$) (Fig. 1). This decline was largely due to changes in cladoceran zooplankton, which decreased on average by four species in invaded lakes ($t = -3.28$, $p < 0.01$) (Fig. 1). Copepod richness was similar in invaded and reference lakes ($t = 0.76$, $p = 0.46$) (Fig. 1). There was no difference in Shannon–Wiener diversity in total zooplankton ($t = -1.31$, $p = 0.22$) and copepods ($t = -0.40$, $p = 0.70$) in invaded lakes compared with uninvaded lakes, but there was a significant reduction in the diversity of cladoceran zooplankton ($t = -2.17$, $p = 0.05$) (Fig. 1). Evenness of the total zooplankton community ($t = 0.40$, $p = 0.70$) was unaffected by *B. longimanus* invasion, whereas cladocerans experienced a slight increase with invasion ($t = 1.89$, $p = 0.08$), and copepods showed a slight decrease in the *B. longimanus* lakes ($t = -1.89$, $p = 0.08$) (Fig. 1). Invasion had a negative effect on overall zooplankton ($t = -1.91$, $p = 0.08$) and copepod abundance ($t = -1.91$, $p = 0.08$), resulting in a twofold difference for both groups. In addition, there was a decline in cladoceran abundance in invaded lakes ($t = -2.86$, $p = 0.01$), where an almost fourfold difference was observed (Fig. 1).

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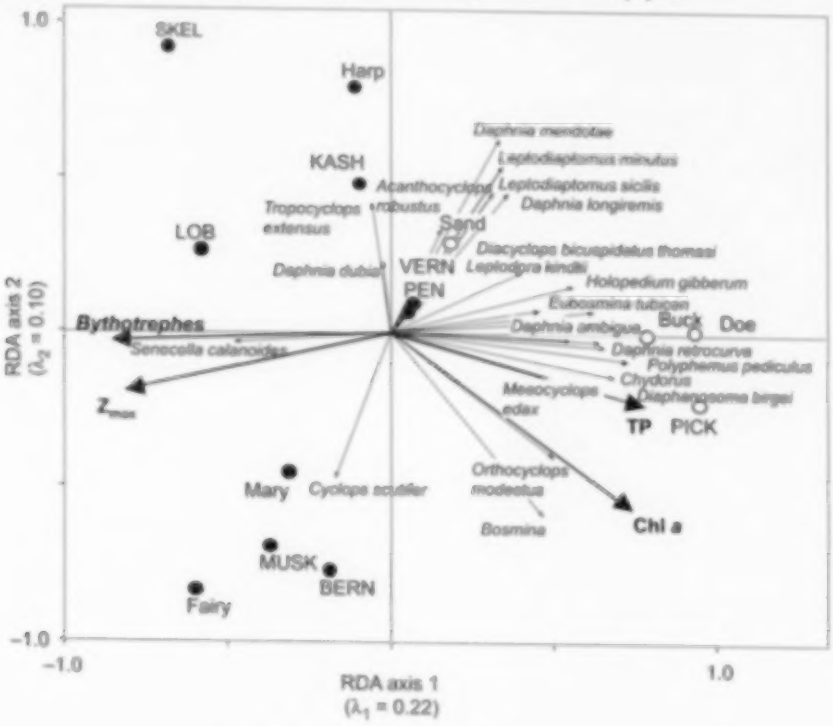
Fig. 3. Redundancy analysis of zooplankton species abundances and environmental conditions. Environmental variables are represented by the large arrows and species are represented by the small arrows. Species with short arrows were removed to reduce crowding. Circles are site scores for invaded (●) and reference (○) lakes. Species names are italicized. Abbreviated lake names: BERN, Bernard; KASH, Kashagawigamog; LOB, Lake of Bays; MUSK, Lake Muskoka; PEN, Peninsula; PICK, Pickerel; SKEL, Skeleton; VERN, Vernon. Environmental variables: TP, total phosphorus; Z_{max}, maximum depth; chl *a*, chlorophyll *a*.



The relationship between *B. longimanus* abundance and zooplankton species richness and abundance was inconsistent across the invaded study lakes (Fig. 2). The deviations in species richness and abundance from the reference lake condition generally increased with higher abundance of *B. longimanus*, but some invaded lakes actually had greater richness and abundance than uninvaded lakes. When we explicitly considered changes in zooplankton communities relative to the abundance of *B. longimanus* in the lakes using linear regression, we found that crustacean zooplankton species richness (r^2 = 0.04, p = 0.52), diversity (r^2 < 0.01, p = 0.93), and abundance (r^2 = 0.06, p = 0.42) were not related to the mean abundance of *B. longimanus*. Likewise, cladoceran species richness (r^2 = 0.13, p = 0.20), diversity (r^2 = 0.11, p = 0.26), and abundance (r^2 = 0.15, p = 0.14) were not related to mean *B. longimanus* abundance.

RDA captured a significant amount of variance (p < 0.01) in zooplankton species abundances, with *B. longimanus* presence or absence explaining 43% of total species variation, and Z_{max} (22%), chl *a* (20%), and TP (15%) contributing to the remainder of species abundance variation (Fig. 3). The first axis (λ_1 = 0.22, p = 0.03) contrasts the presence with the absence of *B. longimanus*, as most of the invaded lakes fall on the same side as the *B. longimanus* vector, and all of the reference lakes are on the opposite side of the gradient. A number of species were negatively associated with the invader (e.g., *D. birgei*, *Mesocyclops edax*, *Holopedium gibberum*), and only one species, the glacial relict *Senecella calanoides*, was positively correlated with *B. longimanus* presence. The second axis (λ_2 = 0.10) seems to be related to lake productivity, as some cladoceran taxa (*Bosmina* (*Bosmina*) spp., *Daphnia retrocurva*, and *D. birgei*) and omnivorous cyclopoids (*M. edax* and *Orthocyclops modestus*) were positively associated with the TP and chl *a* vectors.

The majority of zooplankton taxa were, on average, in lower abundance in invaded lakes than in reference lakes (Fig. 4). The cladoceran taxa tended to fall further below the 1:1 line than did the copepods, especially smaller-bodied taxa such as *Chydorus* spp. and *Eubosmina coregoni*. A few larger taxa, such as *S. calanoides*, *Cyclops scutifer*, and *Daphnia dubia*, were more abundant in invaded lakes than reference lakes, on average, but for the most part, both large- and small-bodied zooplankton were less abundant in *B. longimanus* invaded lakes.

Species richness tended to increase with longer persistence times of *B. longimanus* populations throughout the summer (r^2 = 0.31, p = 0.09), suggesting that there is no temporal refuge available to zooplankton. Using multiple regression analysis, we found that all of the most parsimonious models to explain species richness had negative associations of species richness with mean *B. longimanus* abundance, but that zooplankton richness was positively re-

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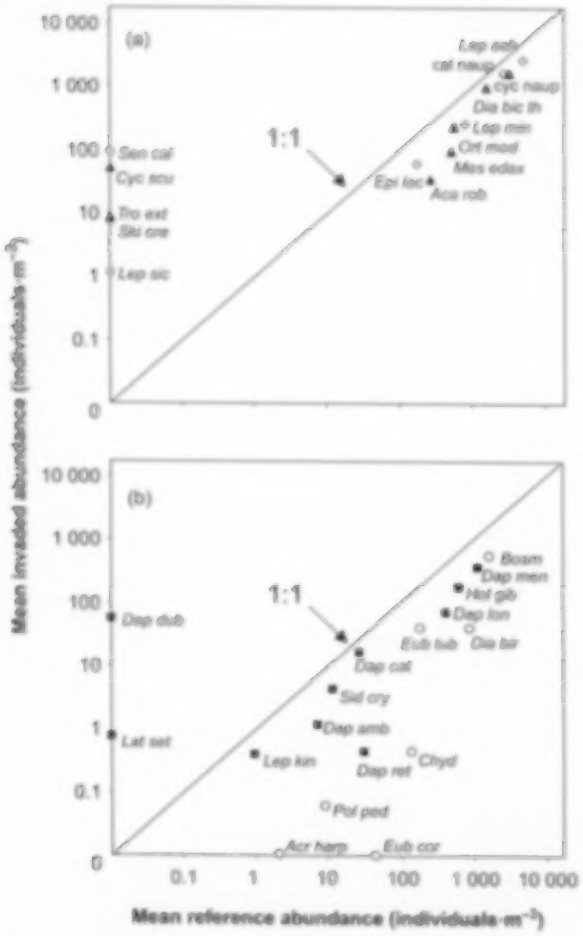
Fig. 4. Mean abundance (individuals·m^{-3}) of (*a*) calanoid (○) and cyclopoid (▲) copepods and (*b*) large (■; >1 mm) and small (○; <1 mm) cladocerans in reference lakes plotted against corresponding abundance in invaded lakes. Abbreviations of zooplankton taxa: (*a*) *Aca rob, Acanthocyclops robustus*; cal naup, calanoid nauplii; cyc naup, cyclopoid nauplii; *Cyc scu, Cyclops scutifer*; *Dia bic th, Diacyclops bicuspidatus thomasi*; *Epi lac, Epischura lacustris*; *Lep ash, Leptodiaptomus ashlandi*; *Lep min, Leptodiaptomus minutus*; *Lep sic, Leptodiaptomus sicilis*; *Mes edax, Mesocyclops edax*; *Ort mod, Orthocyclops modestus*; *Sen cal, Senecella calanoides*; *Ski ore, Skistodiaptomus oregonensis*; *Tro ext, Tropocyclops extensus*; (*b*) *Acr har, Acroperus harpae*; *Bosm, Bosmina* (*Bosmina*) spp.; *Chyd, Chydorus* spp.; *Dap amb, Daphnia ambigua*; *Dap cat, Daphnia catawba*; *Dap dub, Daphnia dubia*; *Dap lon, Daphnia longiremis*; *Dap men, Daphnia mendotae*; *Dap ret, Daphnia retrocurva*; *Dia bir, Diaphanosoma birgei*; *Eub cor, Eubosmina coregoni*; *Eub tub, Eubosmina tubicen*; *Hol gib, Holopedium gibberum*; *Lat set, Latona setifera*; *Lep kin, Leptodora kindtii*; *Pol ped, Polyphemus pediculus*; *Sid cry, Sida crystallina*.



lated to *B. longimanus* persistence in high abundances in the lake (Table 3). In both models, species richness was positively related to duration of *B. longimanus* persistence and TP and negatively related to mean *B. longimanus* abundance. In addition, the best model selected by AIC had positive associations of zooplankton richness with area and pH. The presence of fish planktivore species had no effect on zooplankton species richness.

Discussion

Our study has demonstrated that *B. longimanus* can have negative effects on zooplankton communities through parts of the ice-free season, reducing species richness, diversity, and abundance throughout the ice-free season. This negative effect was exacerbated in the cladocerans, which showed significant declines in richness (on average, four less species in invaded lakes), abundance (a fourfold decline), and diversity. These results are in agreement with the declines in zooplankton richness observed in Harp Lake (Yan et al. 2002), Muskoka lakes (Boudreau and Yan 2003), and Lakes Michigan, Erie, and Huron (Barbiero and Tuchman 2004) (Table 4). Reductions in the abundance of zooplankton have been shown experimentally (Strecker and Arnott 2005) and in Harp Lake (Yan et al. 2001); however, some recent studies have failed to detect changes in total zooplankton community abundance (Boudreau and Yan 2003; Barbiero and Tuchman 2004) (Table 4). The samples in Boudreau and Yan's (2003) study were taken on a single sampling date, and this snapshot approach does not take into account seasonal dynamics, as was done in our study and Yan et al. (2001). Barbiero and Tuchman's (2004) samples were only taken for 1 month starting 1 August and, thus, do not reflect zooplankton abundance in late spring, early summer, and fall. A comparison of our study with others over a shorter period of time suggests that short-term synoptic surveys may adequately document changes in some measures of zooplankton community structure associated with *B. longimanus* invasion. For example, the effect of *B. longimanus* on species richness seems to be consistent between different sampling routines, but longer studies, especially in smaller inland lakes, that encompass the entire season seem to capture changes in total abundance better than single sampling dates (Table 4).

Several zooplankton taxa showed steep declines in *B. longimanus* invaded lakes. As past studies have documented the general negative effect that *B. longimanus* has on many cladoceran taxa (Boudreau and Yan 2003), we expected to observe this in our study as well. As expected, the abundances of a number of common cladoceran species, such as *Bosmina* (*Bosmina*) spp., and *D. birgei*, were severely reduced: on average, a decrease of greater than four times in invaded lakes. The larger species *Daphnia longiremis*, *Daphnia mendotae*, and *H. gibberum* also had lower abundances in invaded lakes, suggesting that body size alone does not provide refuge from predation. As well, it appears as though some hypolimnetic species, such as *D. longiremis* (Keller and Conlon 1994), do not escape predation. These patterns are generally in agreement with Boudreau and Yan (2003), Yan et al. (2001), and Barbiero and Tuchman (2004), although there seem to be some system-specific differences. For example, *D. mendotae* has remained unaffected by *B. longimanus* in Harp Lake (Yan et al. 2001; Boudreau and Yan 2003; this study) but has declined in other lakes (Barbiero and Tuchman 2004). Unexpectedly,

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Table 3. Multiple regression of crustacean zooplankton species richness with predictor variables in all study lakes (invaded: $n = 10$; reference: $n = 4$).

	Adjusted R^2	Mallows C_p	AIC	F ratio	p value	Variable	Coefficient	Partial correlation coefficient	t value	p value
Model 1	0.73	4.59	16.37	12.57	<0.01	*B. longimanus* abundance	−1.32	−0.84	−4.87	<0.01
						B. longimanus persistence	1.23	0.83	4.77	<0.01
						TP	−0.47	0.70	3.12	0.01
Model 2	0.77	5.10	14.77	9.79	<0.01	Area	0.24	0.49	1.57	0.15
						B. longimanus abundance	−1.78	−0.89	−5.65	<0.01
						B. longimanus persistence	1.70	0.89	5.52	<0.01
						pH	0.20	0.43	1.36	0.21
						TP	0.62	0.81	3.93	<0.01

Note: Persistence is defined as number of consecutive sampling dates that *B. longimanus* abundance was >5 individuals·m^{-3}. AIC, Akaike's information criterion; TP, total phosphorus.

Table 4. Summary of effects of *B. longimanus* on several zooplankton community attributes in different types of sampling regimes.

Variable	Long-term study of Harp Lake,[a,b] Great Lakes[c]	Synoptic survey[d]	Seasonal survey[e]	Field experiment[f]
Richness				
Total zooplankton	—[a,c]	—	–	0
Cladocerans	—[a]	—	—	0[g]
Copepods	0[a]	0	0	0[g]
Abundance				
Total zooplankton	–[b], 0[c]	0	–	—
Cladocerans	—[b], 0[c]	—	—	– (small); 0 (large)
Copepods	0[b,c]	0	–	– (calanoids); 0 (cyclopoids)
Diversity				
Total zooplankton	N/A	N/A	0	0
Cladocerans	N/A	N/A	—	0[g]
Copepods	N/A	N/A	0	+[g]

Note: Symbols: +, positive effect ($p < 0.05$); —, negative effect ($p < 0.05$); –, negative effect ($p < 0.1$); 0, no effect.
[a]Yan et al. (2002); biweekly or monthly collections (1980–2000).
[b]Yan et al. (2001); biweekly or monthly collections (1980–1998).
[c]Barbiero and Tuchman (2004); multiple stations in Lakes Michigan, Huron, and Erie (1983–1999).
[d]Boudreau and Yan (2003); single midsummer sampling date (2001).
[e]This study.
[f]Strecker and Arnott (2005); 30-day enclosure experiment.
[g]A. Strecker, unpublished data.

several common copepod taxa (*Leptodiaptomus minutus*, *Diacyclops bicuspidatus thomasi*, and *M. edax*) in our study were also negatively affected by *B. longimanus*. Previously, only *M. edax* was thought to be affected by invasion (Boudreau and Yan 2003; Barbiero and Tuchman 2004). Given that copepods are in high abundance in the late spring in these lakes, before *B. longimanus* emerges from resting eggs and rapidly builds up populations through parthenogenic reproduction (A. Strecker, unpublished data), it is especially surprising that they declined, as their earlier dominance in lakes would suggest that they should be able to use a temporal refuge from *B. longimanus* predation. However, longer-lived slow-reproducing copepods may actually be more susceptible to predation by *B. longimanus* because they are unable to reproduce as quickly as cladocerans. Thus, the copepods are less able to respond to low predator abundances, as *B. longimanus* is known to have extremely patchy spatial and temporal dynamics (J. Young and E. Parrott, Department of Biology, York University, Toronto, ON M3J 1P3, Canada, unpublished data) and, therefore, cannot take advantage of this window of opportunity. This further supports the notion that to adequately assess changes in total and individual zooplankton abundances resulting from *B. longimanus* invasion, longer-term surveys are necessary.

Our study suggests that most species are not able to take advantage of a temporal refuge from *B. longimanus* predation. We found that longer persistence times of *B. longimanus* resulted in higher species richness, the opposite of what we would expect to see if a temporal refuge was indeed available. *Bythotrephes longimanus* was detected in invaded lakes on an average of seven sampling dates, or about 14 weeks during the summer. This is likely a conservative estimate, as *B. longimanus* was present in 90% of invaded lakes on the final sampling date in September and has shown the ability to persist into late fall in some lakes (J. Young, Department of Biology, York University, Toronto, ON M3J 1P3, Canada, personal communication). Thus, in most lakes, the only op-

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portunity for a temporal refuge is in the spring, and our evidence suggests that this window of opportunity is not being used by zooplankton, perhaps because water temperatures are cool and food availability is low in these oligo-mesotrophic lakes. If zooplankton were indeed using this temporal refuge, we would expect to see no effect of *B. longimanus* on zooplankton richness and abundance, especially on copepods, which are typically in high numbers in these lakes in late spring, thus having reduced temporal overlap with the invader. However, this was not the case: overall zooplankton abundance and richness were reduced, and several copepod taxa were also negatively affected by *B. longimanus*. Another potential explanation is that there are competitive interactions occurring between *B. longimanus* and its predators. *Mysis relicta* and other zooplanktivores may benefit from longer *B. longimanus* persistence times by switching from smaller zooplankton to the larger invader, thus freeing the zooplankton from intense predation. Gut content analysis of *M. relicta* collected from several of our study lakes has revealed the presence of *B. longimanus* body parts, thus it seems that the invader can also be a prey item for other invertebrate predators (Nordin 2005).

There was a substantial amount of among-lake variation in the responses of the zooplankton communities to *B. longimanus* invasion. This variability appears to be unrelated to the actual abundance of *B. longimanus*, which was not a strong predictor of zooplankton richness or abundance in invaded lakes. There is some evidence that zooplankton can adapt their morphologies in the presence of *B. longimanus* (Bungartz and Branstrator 2003), suggesting that the abundance of the invader may play a role in eliciting behavioural and morphological responses in the zooplankton community and thus increasing species survival. Two of the invaded lakes, Peninsula and Vernon, had zooplankton communities that more closely resembled communities in non-invaded reference lakes. Both lakes had relatively high mean *B. longimanus* abundance (7.8 and 4.3 individuals·m^{-3}, respectively) and had richness values that fell more into the range of uninvaded lakes. When these lakes were excluded from regression analyses, mean *B. longimanus* abundance had a significant negative effect on species richness (r^2 = 0.53, $p < 0.01$). The redundancy analysis also suggests that Peninsula and Vernon lakes have species compositions that are more similar to those of reference lakes than invaded lakes. We are unsure why the zooplankton community composition of these two lakes is seemingly unaffected by invasion. One possibility is that *B. longimanus* is feeding in a density-dependent manner, reducing the abundances of certain preferred species to low densities, and then switching to higher-density prey items that can be caught more easily. Thus, *B. longimanus* is not reducing any species to the point where it can no longer be detected. Consistent with this hypothesis, the evenness of Peninsula and Vernon lakes was higher than the average of the invaded lakes, and the diversity was greater than the average diversity of the uninvaded lakes. The abundances of the zooplankton communities of both of these lakes are below the average of other invaded lakes, so although it seems that *B. longimanus* is reducing zooplankton abundance, species richness remains unaffected. However, if *B. longimanus* feeding is indeed density-dependent in these lakes, then why is this not occurring in all of the invaded lakes? It is possible that zooplankton reproduction in these lakes was high enough to fend off the loss of species richness. Yan et al. (1991) showed that for the rotifer *Keratella tauroscephala*, high rates of production could be maintained, even under intense predation by species of the invertebrate *Chaoborus*. Vernon Lake had high secondary production relative to *B. longimanus* consumption (A. Strecker, unpublished data), so this may, in part, explain the lack of effect on species richness.

Other potential explanations for the high levels of species richness observed in Peninsula and Vernon lakes are that they had different morphological, biotic, and chemical conditions than the rest of the invaded lakes, and thus, there existed some advantage that allowed coexistence. For instance, a deep-water spatial refuge in the lake can afford some species a certain amount of protection from predation. However, Peninsula and Vernon lakes were the two shallowest invaded lakes, suggesting that they, in fact, had a smaller spatial refuge than other invaded lakes. Another possibility is that Peninsula and Vernon lakes were less clear than other invaded lakes, thus offering zooplankton a dark-water refuge. This may be the case for Vernon Lake, which had higher DOC and lower Secchi disc values than did other invaded lakes, but not for Peninsula Lake, which had values that were within the range of the other invaded lakes. Thus, this variability that we observed between study lakes in response to an invasion of *B. longimanus* may be due to a combination of factors related to characteristics of the invader, i.e., feeding strategy, and to characteristics of the lakes, such as productivity and lake clarity.

The importance of lake area and productivity in determining crustacean zooplankton species richness in temperate freshwater lakes has been well established (Dodson 1992; Dodson et al. 2000). Surface area had little predictive power in our analysis of factors influencing species richness in lakes invaded by *B. longimanus*, but other variables related to the invader were much better predictors of zooplankton richness. Although we did not measure primary productivity, TP can be used as a surrogate for productivity (see Leibold 1999). A caveat of our study is that uninvaded lakes had slightly higher levels of TP and chl *a* than did the invaded lakes, but in our oligo-mesotrophic systems, these differences are small and are unlikely to lead to the observed differences in species richness. In addition, our multiple regression analyses suggest that although TP is an important predictor, *B. longimanus* explains far more variation in species richness. Although *B. longimanus* abundance by itself was a poor predictor of species richness, when combined with persistence of the invader in multiple regression analysis, the explanatory power of the two variables was much greater. It is possible that the result from the linear regression was being influenced by the two outlier values, Vernon and Peninsula lakes, while these values became less important with more predictive variables. In fact, neither of these values had an exceptionally large influence on the final multiple regression models, as measured by Cook's distance statistic (Quinn and Keough 2002).

The composition of the fish community can also have substantial effects on the structure of the zooplankton community (Brooks and Dodson 1965). Although there appear to be some differences in the abundance of planktivores present in

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invaded versus reference lakes, our data suggest that *B. longimanus* is still having a substantial negative effect. If fish were in fact responsible for the effects that we observed, then we would expect larger zooplankton species to be more negatively affected than small taxa. However, reductions in species richness were proportionately greater for small species (28%) than for large-bodied zooplankton (14%), which supports our assertion that *B. longimanus* is largely responsible for the observed reduction in zooplankton species richness. Other variables, such as pH (Keller and Pitblado 1984), calcium (Schell et al. 2001; Wærvågen et al. 2002), and postglacial history of the lake (Dadswell 1974; Carter et al. 1980), can be important predictors of zooplankton richness. However, neither pH nor calcium were significantly different in *B. longimanus* lakes compared with reference lakes, and all of our study lakes have a similar postglacial history because of the presence of *M. relicta*. Thus, these factors did not influence our results.

There is now a significant body of evidence to suggest that *B. longimanus* invasions cause changes to the zooplankton communities of freshwater lakes. This is especially true of cladoceran taxa, which seem to take the brunt of *B. longimanus* predation, as our study has exhibited striking declines in their richness, abundance, and diversity in invaded lakes. A concurrent study of the rotifer composition and abundance of the same 14 lakes showed a positive relationship between rotifer and *B. longimanus* abundance, perhaps as a result of reduced competition with herbivorous cladocerans for phytoplankton prey (Hovius et al. 2006). These results are somewhat surprising, as *B. longimanus* is an invertebrate predator that has several native functional analogues in these boreal lakes, including *M. relicta*, *Leptodora kindtii*, *Chaoborus* spp., and water mites (Arachnida, Hydrachnidia). It could be argued that *B. longimanus* occupies a unique feeding niche; however, this seems unlikely as its dietary overlap with other invertebrate predators is high (McNaught et al. 2004; Young and Riessen 2005). *Bythotrephes longimanus* employs several effective strategies to maintain high feeding rates in these boreal lakes: (*i*) although it is consumed by some fish species, presumably the long tail spine of *B. longimanus* decreases predation impact; (*ii*) it lacks large amplitude migrations (Yan and Pawson 1997), unlike many other invertebrate predators (*Chaoborus*, *Mysis*, *Leptodora*), so it is continuously in regions of high prey density; (*iii*) it reproduces parthenogenically, unlike mites, which are sexual and have slower reproductive times, so it can quickly respond to increases in food resources; and (*iv*) its neonates are larger, allowing them to exploit a larger range of prey sizes, unlike the small-bodied clutches of *Leptodora* (Branstrator 2005). What is clear is that not all lakes respond to *B. longimanus* invasion in the same way. Our study has highlighted that there are substantial among-lake differences in the effect of the invader on richness, abundance, and diversity of zooplankton communities.

As *B. longimanus* continues to spread across the landscape of North America, it seems likely that it will be an important new factor in determining the zooplankton composition of freshwater lakes. This is certain to have implications for regional species richness, as our results suggest that certain species, such as the ubiquitous taxa *D. birgei* and *M. edax*, have reduced abundances in invaded lakes across the landscape, leaving few populations that may act as sources to disperse to new locales.

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Are there hot spots for *Elliptio complanata* in the shallow littoral zone of a large Canadian Shield lake?

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Abstract: The variability in habitat conditions in large lake basins may provide areas favourable for unionid recruitment and growth. Here we test whether the distribution, body size, and growth rate of *Elliptio complanata* differ between upwind and downwind sites in a 5.8 km long lake basin. We measured density and morphological characteristics of *E. complanata* at nine upwind and nine downwind sites. We also measured growth rates and stable isotope ($\delta^{13}C$, $\delta^{15}N$) signatures at three upwind and three downwind sites. We found no difference in density or body size of *E. complanata* between upwind and downwind sites, despite clear differences in water temperature and food availability. Contrary to our expectations, the growth rate of *E. complanata* was higher (by 62%) at upwind sites. The reason for this difference is uncertain, but higher shell erosion and a negative relationship between $\delta^{13}C$ and C/N ratio suggest that *E. complanata* may be more stressed at downwind sites. The $\delta^{15}N$ of *E. complanata* did not differ systematically between upwind and downwind sites, but increased with increasing body size. It would therefore be prudent to standardize the size of *E. complanata* used to measure the baseline of lake food webs.

Résumé : La variabilité des conditions d'habitat dans les grands bassins lacustres peut créer des zones favorables au recrutement et à la croissance des unionidés. Nous vérifions ici si la répartition, la taille corporelle et le taux de croissance d'*Elliptio complanata* diffèrent entre les sites au vent et les sites sous le vent dans un bassin lacustre de 5,8 km de longueur. Nous avons mesuré la densité et les caractéristiques morphologiques d'*E. complanata* à neuf sites au vent et à neuf sites sous le vent, de même que les taux de croissance et les signatures d'isotopes stables ($\delta^{13}C$, $\delta^{15}N$) à trois sites au vent et trois sites sous le vent. Il n'y a pas de différence de densité ni de taille chez *E. complanata* entre les sites au vent et sous le vent, malgré des différences évidentes de température de l'eau et de disponibilité de nourriture. Contrairement à nos prévisions, le taux de croissance d'*E. complanata* est plus élevé (de 62 %) aux sites au vent. La raison de cette différence est incertaine, mais l'érosion plus grande de la coquille et la relation négative entre $\delta^{13}C$ et le rapport C/N laissent croire qu'*E. complanata* est peut-être plus stressé aux sites sous le vent. Il n'y a pas de différence systématique de $\delta^{15}N$ chez *E. complanata* entre les sites au vent et sous le vent, mais $\delta^{15}N$ augmente en fonction de la taille du corps. Il serait donc prudent de standardiser la taille d'*E. complanata* pour établir les valeurs de base danétude des réseaux trophiques des lacs.

[Traduit par la Rédaction]

Introduction

In some freshwater systems, unionids dominate the benthos, accounting for up to 90% of the biomass of benthic invertebrates (Negus 1966; Strayer et al. 1999). Because of their dominating presence in the benthos, unionids may exert strong effects on aquatic ecosystem processes (Strayer et al. 1999; Vaughn and Hakenkamp 2001). For instance, unionids are important filter feeders (filtering up to 10%–100% of the water column per day) and may alter benthic processes by burrowing through sediments and enriching them with organic matter. However, while unionids play an important role in freshwater ecosystems, little is known about the biotic and abiotic factors that affect their growth and spatial distribution. The notorious heterogeneity of littoral habitats in lakes may provide hot spots for unionid productivity.

In Lake Opeongo, a large Canadian Shield lake, the prevailing winds blow along a 5.8 km long basin, creating different habitat conditions at upwind and downwind sites. The downwind end of the basin is warmer and has more stable water temperature (Finlay et al. 2001). It also receives higher plankton biomass from wind-driven currents (Kaevats et al. 2005; A. Blukacz and W.G. Sprules, Department of Zoology, University of Toronto at Mississauga, Mississauga, ON L5L 1C6, personal communication). Increased water temperatures may increase metabolic rates of exothermic unionids and may lengthen their growth season, potentially leading to increased growth. Increased plankton densities

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may also positively affect unionid growth through bottom-up effects. This suggests that the littoral zone on the downwind end of the basin should provide a more favourable habitat for unionids.

The feeding regimes of unionids may also differ between upwind and downwind sites because of changes in food sources. For instance, wind blowing across a lake may transport different planktonic organisms (i.e., phytoplankton, zooplankton) differently to downwind sites or may resuspend benthic organisms at these sites and thus may alter the feeding regime of downwind versus upwind unionids. Stable isotopes of ^{13}C and ^{15}N may be used to test these potential changes in energy flow. Carbon isotope ratios ($\delta^{13}C$) are used to construct feeding relationships, as the $\delta^{13}C$ signature of an organism is similar to that of its food source (Wada et al. 1993; Post 2002). In freshwater lakes, phytoplankton and benthic algae have distinct $\delta^{13}C$ signatures, with benthic algae showing less ^{13}C discrimination during carbon fixation ($\delta^{13}C$ benthic algae: –26‰; phytoplankton: –32‰; France 1995*a*). This difference in the primary producers is reflected in consumers (France 1995*b*; Vander Zanden and Rasmussen 1999). The nitrogen isotope ratio ($\delta^{15}N$) is used to determine the trophic position of an organism, as consumers are enriched in ^{15}N by 2.5‰–3.4‰ relative to their prey (Minagawa and Wada 1984; Post 2002; Vanderklift and Ponsard 2003). The $\delta^{15}N$ signature of mussels is assumed to remain relatively constant with increasing body size. This was shown by Minagawa and Wada (1984) in two species of marine mussels (*Septifer virgatus* and *Mytilus edulis*) that attach to hard surfaces. However, the distribution of phytoplankton and zooplankton is not homogeneous in lakes (nor in marine coastal areas), and wind-driven currents could transport phytoplankton and zooplankton differently to different parts of the basin, depending on the size and buoyancy of the organisms. Moreover, freshwater unionids live in soft sediments, and young mussels bury into the sediments sometimes for extended periods of time (Amyot and Downing 1991). Systematic differences in food sources available in different parts of the lake basin and in the timing of burial among unionids of different sizes could affect their $\delta^{15}N$ signature and their reliability as a food web baseline.

In this study, we test whether the density, body size, and growth rate of the unionid *Elliptio complanata* differ with habitat variability (specifically temperature and food availability) at upwind and downwind sites in Lake Opeongo. We also investigate differences in trophic relationships between upwind and downwind sites by comparing their stable isotope signatures ($\delta^{13}C$ and $\delta^{15}N$).

Materials and methods

Site description

Lake Opeongo is a multibasin oligotrophic lake located on the Canadian Shield in Algonquin Park, Ontario, Canada (45°42′N, 78°22′W). This study was done in South Arm, a 5.8 km long basin, which is oriented NW–SE along one of the predominant wind axes (Fig. 1). The western end of the basin is usually upwind and the eastern end usually downwind, and we will refer to these as "upwind" and "downwind", respectively. Wind events in this basin result in strong internal waves that influence water temperature in different parts of the littoral zone (Finlay et al. 2001).

Water temperature at upwind and downwind sites

We compared water temperature at two upwind and two downwind sites (U3, U7, D7, and D9 in Fig. 1) using Stowaway temperature loggers (models STEB08, STEB16, and TBI32-05+37, Hoskin Scientific, Burlington, Ontario) from May to September–October in three consecutive years (1998–2000). The temperature loggers were placed 20–40 cm above the substrate in the shallow littoral zone (1–2.5 m depth) on cement blocks. The loggers were programmed to record water temperature at 8–16 min intervals simultaneously at all sites. All temperature loggers were calibrated with a mercury thermometer at room temperature and at 4 °C, at the beginning and (or) the end of each field season (see details in Finlay et al. 2001).

Sampling of planktonic organisms

We compared phytoplankton biomass at upwind and downwind sites after two wind events: 17 June and 25 June 2004. Six litres of lake water were collected just below the surface at nine inshore sites around South Arm (site depths = 0.5–0.75 m, within 5 m from shore) and at one offshore site (triangles in Fig. 1). Three replicate samples were filtered on GF/F filters, ground with a mortar and pestle, and extracted overnight in 95% ethanol. The samples were centrifuged and the supernatant was read on a Milton Roy Spectronic 1001+ spectrophotometer (Milton Roy Company, Rochester, New York) at 665 nm (±2 nm). Chlorophyll concentrations were corrected for phaeopigment interference by acidifying with 0.2 mol·L^{-1} HCl (Lorenzen 1967).

Planktonic organisms were also sampled concurrently with unionids (29 September 2004) for stable isotope analysis at one upwind and one downwind offshore site (stars in Fig. 1). At each site, the plankton sample consisted of the pooled contents of five 10 m vertical tows of a 63 µm mesh Wisconsin net.

Field collection of *E. complanata*

Elliptio complanata were collected at nine upwind and nine downwind sites within South Arm (open and solid circles, respectively, in Fig. 1) on 28 and 29 September 2004. The water temperature was 17 °C at the time of sampling. At each site, a 1 m^2 quadrat was thrown into the water at ~2 m depth. All unionids in the quadrat were collected, and the substratum was searched by hand for unionids buried within the sediment. The density of unionids and the substratum type were recorded at each site. Twenty-five unionids were collected at three upwind (U3, U7, U9) and three downwind (D4, D8, D9) sites (for a total of 150) for morphological characterization and growth rate measurement.

Morphological characteristics

Upon return to our field laboratory, the unionids were placed in an aquarium of filtered lake water for 24 h to clear their gut. Soft tissues were then removed from each unionid and the guts were cleaned of remaining material with filtered lake water. The cleaned tissues were placed into a pre-weighed aluminum dish and dried at 60 °C for 48 h. Soft

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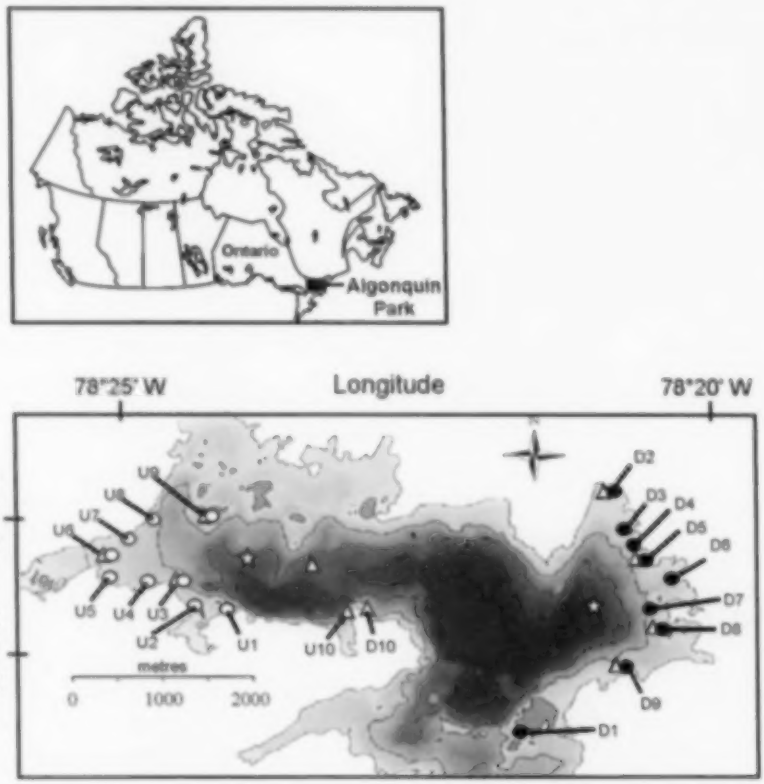
Fig. 1. Sampling sites in the South Arm of Lake Opeongo (Algonquin Park, Ontario, Canada). *Elliptio complanata* mussels were sampled at nine upwind sites (open circles, U1–U9) and nine downwind sites (solid circles, D1–D9). Planktonic organisms were sampled for stable isotope analysis at two offshore sites (stars): one upwind and one downwind. To compare the potential availability of planktonic food upwind and downwind, chlorophyll concentrations were measured at nine inshore sites and one offshore site after two wind events (triangles). The bathymetric contour lines are at 10 m intervals.



tissue mass was then measured to 0.0001 g using a Mettler AE50 balance (Mettler–Toledo Inc., Columbus, Ohio).

We measured the growth length (from the umbo to the point of maximum growth), maximum length, maximum width, and maximum depth of *E. complanata* shells using calipers (±0.01 mm). We also measured the length of shell that was eroded along the growth length. The proportion of the growth length that was eroded is used as an index of shell erosion. Shells were then dried at 60 °C for 24 h, after which the mass was measured to 0.01 g on a Mettler PB3002 balance. Shell thickness was calculated as the ratio of shell mass to maximum length (Bailey and Green 1987).

Growth rate

The growth rates of unionids were determined by measuring the distances between the last three growth lines laid in the shells. Unionid shells were thin-sectioned following procedures described by Day (1984) and Neves and Moyer (1988). Shells were cut along the growth axis using a Raytech Jemsaw 45 (Raytech Industries, Middletown, Connecticut). The cut surface was polished first with a belt sander and then with a series of fine-grit sandpapers (120–2000 grit), until the shell lay perfectly flat on a microscope slide. The cut side was then mounted in 5 min epoxy onto a microscope slide and dried at 60 °C for 24 h. The remaining shell was sawed off using a DeWalt cutter and grinder 10-40 (DeWalt, Baltimore, Maryland), leaving a 1 mm thick section. This section was then polished using very fine-grit sandpapers (1200 and 2000 grit) to a thickness of ~250 µm or a desired transparency in which growth lines could be observed.

Thin sections were viewed under a light microscope (Leica DM4500) at 10× magnification, and the distances between the last three growth lines (laid nearest to the growth edge) were measured using an image analysis program (Open Lab 4.0.2; Improvision, Lexington, Massachusetts). We could only measure three growth periods in most unionids, since large portions of the prismatic layer were eroded. Following Veinott and Cornett (1996), opaque bands were considered growth lines if they extended from the prismatic layer discontinuity to the umbo or if the opaque band extended from the prismatic layer and merged into a line common to several opaque bands (Fig. 2). Growth was measured for 9–15 individuals per site (D4, $n = 15$; D8, $n = 13$; D9, $n = 9$; U3,

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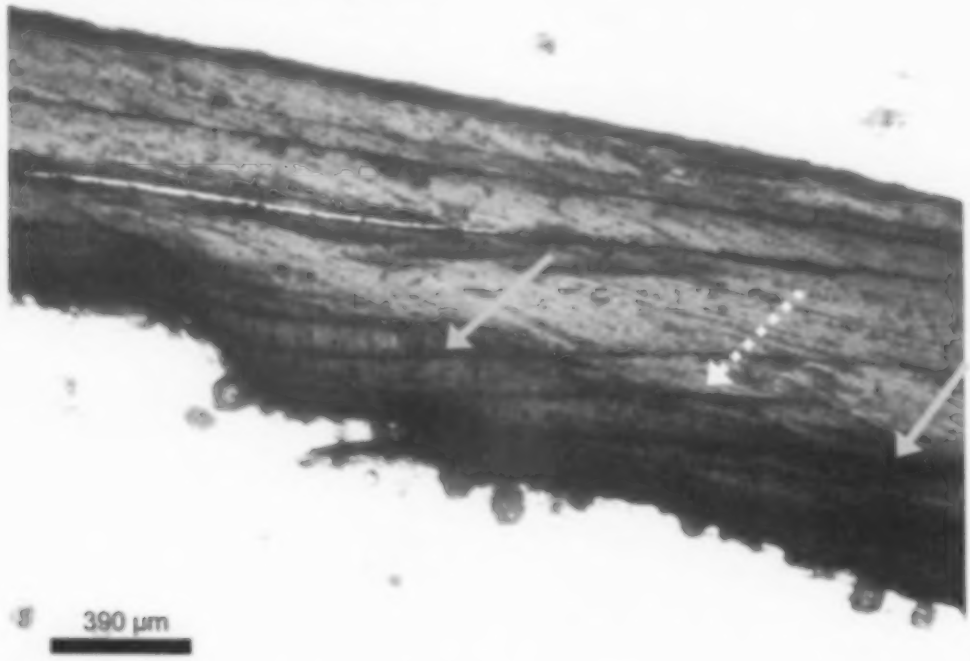
Fig. 2. An *Elliptio complanata* thin-sectioned shell (JB9 #2) viewed under a light microscope at 10× magnification. Solid arrows are pointing to growth lines found near the tip of the shell, which extended from the prismatic layer and merged into a line common to several opaque bands (merge not shown). The broken arrow-points to a line that extended from the prismatic layer, but did not merge into a common line, and thus was not considered a growth line.



$n = 13$; U7, $n = 13$; U9, $n = 10$). Shells in which the growth edge was damaged by erosion or by thin-sectioning were not measured.

Stable isotope analysis

Unionid soft tissue (whole body) and plankton samples were dried at 60 °C for 48 h and ground using a mortar and pestle. Unionid soft tissue samples from four sites (D4, $n = 2$; D9, $n = 7$; U3, $n = 2$; U7, $n = 7$) and plankton samples from two offshore sites were sent to the Environmental Isotope Laboratory at the University of Waterloo, Ontario, for ^{13}C, ^{15}N, and total N and C analysis. We selected unionids spanning the full range of available body sizes for these analyses to examine the potential relationships between body size and stable isotope signature. Most unionids were selected from two sandy sites (U7, D9) to control for substrate-related variation. Two mussels (one large and one small) were selected from mud (U3) and boulder (D4) substrates to explore differences between substrata.

Statistical analysis

Unionid density was compared between upwind and downwind sites and among substrate types (mud, sand, boulders) using a t test and an analysis of variance (ANOVA) on $\log_{10}(x + 1)$-transformed data (Zar 1984). The body sizes of the unionids were compared between upwind and downwind sites in two ways. We compared the growth lengths of unionids between upwind and downwind sites and among upwind sites and among downwind sites using Kruskal–Wallis tests (Conover 1980). We also used a canonical discriminant analysis based on ln-transformed measures of maximum length, maximum width, maximum height, and shell mass to compare the size of unionids at upwind and downwind sites (Pielou 1984). Because of the large amount of erosion on most shells, we repeated this analysis with measures of linear dimension only (i.e., without shell mass) and found very similar results. Maximum length – dry mass relationships were compared between upwind and downwind sites by analysis of covariance (ANCOVA) on ln-transformed variables, with a dummy variable for site location (Zar 1984). Shell thickness and degree of erosion were also compared between upwind and downwind sites using ANCOVA on ln-transformed variables (Zar 1984). Growth rates in each of the three growth periods were compared between upwind and downwind sites using Kruskal–Wallis tests (Conover 1980). The relationship among stable isotope signatures ($\delta^{13}C$, $\delta^{15}N$), maximum length, and C/N ratios in unionids were tested with simple regression analysis (Zar 1984). All analyses were performed in Statistica 5.0 for microcomputers (StatSoft Inc., Tulsa, Oklahoma).

Results

Environmental differences between upwind and downwind sites

There are clear differences in water temperature between shallow littoral sites at the downwind and the upwind ends of South Arm (Fig. 3). The downwind end is warmer than

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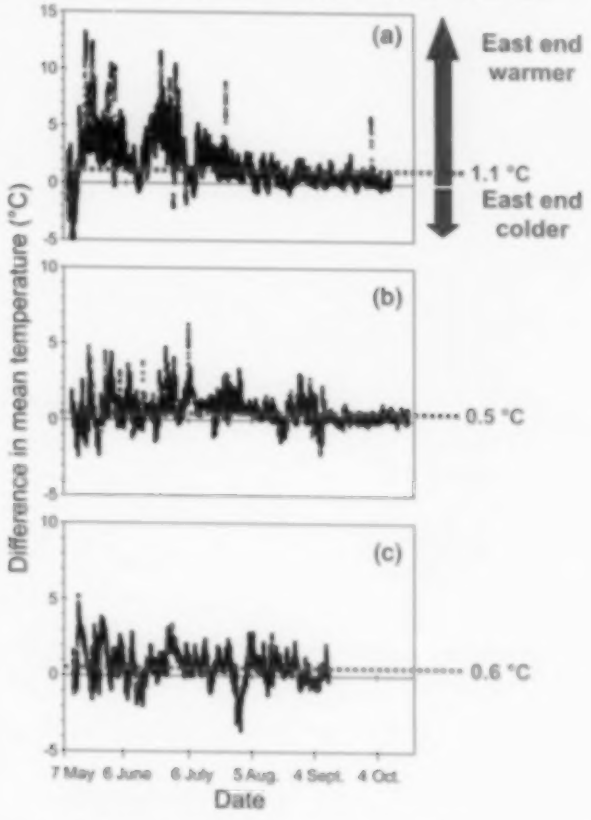
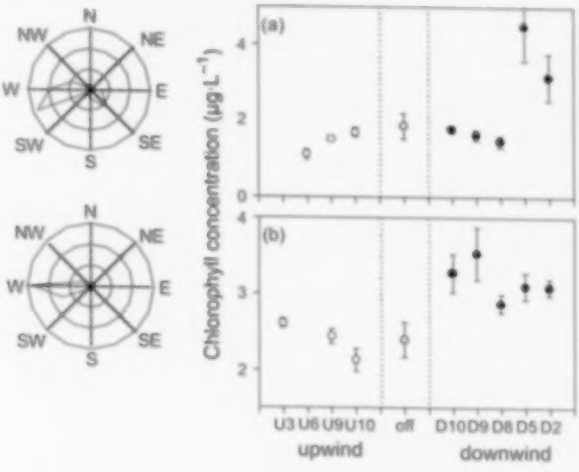
Fig. 3. Difference in mean water temperature between shallow littoral sites at the eastern (downwind) and the western (upwind) end of South Arm in three consecutive years: (*a*) 1998, (*b*) 1999, (*c*) 2000. These differences were calculated from simultaneous measurements of water temperature at two downwind (D7, D9) and two upwind (U3, U7) sites every 8 or 16 min. The eastern end of South Arm was generally warmer then the western end (positive differences). Median differences in mean water temperature over the whole growing season (shown as broken lines) were (*a*) 1.1 °C in 1998, (*b*) 0.5 °C in 1999, and (*c*) 0.6 °C in 2000.



the upwind end by as much as 10 °C, with the most pronounced differences occurring in the spring and early summer. These differences in water temperature between upwind and downwind sites depend on wind and weather patterns, and their magnitude varies among years. Median differences in water temperature between the downwind and upwind ends of the basins were 1.1 °C in 1998, 0.5 °C in 1999, and 0.6 °C in 2000 (Fig. 3). These results extend the findings of Finlay et al. (2001) and suggest that unionids at the downwind end of South Arm are exposed to warmer temperatures, possibly resulting in a longer growing season.

Phytoplankton biomass differed between the upwind and downwind ends of South Arm after two wind events (Fig. 4). Chlorophyll concentrations are typically low in Lake Opeongo, but they ranged more than fourfold (1.1–4.5 $\mu g \cdot L^{-1}$) among sites. The sampling of inshore phytoplankton biomass on 17 June followed a windy night, with winds up to 18 $km \cdot h^{-1}$. In the morning, the chlorophyll concentration inshore was

Fig. 4. Surface chlorophyll *a* concentrations measured at shallow littoral sites and offshore (off) following two wind events: (*a*) 17 June 2004 and (*b*) 25 June 2004. Data points represent the mean chlorophyll concentration ± standard error (n = 3). The solid line on the rosettes (left) show the proportion of the wind that blew from each cardinal directions in the 12 h before each sampling. Sampling sites are shown as triangles in Fig. 1.



lowest (1.1 $\mu g \cdot L^{-1}$) at the two most upwind sites (U3 and U6), and we found >50% higher chlorophyll concentration offshore and at sites U10 and D10 in the center of the basin (1.7–1.9 $\mu g \cdot L^{-1}$). The highest chlorophyll concentrations were measured at downwind sites D2 and D5 (3.2–4.5 $\mu g \cdot L^{-1}$), both located in an embayment on the northern shore (Fig. 1). Phytoplankton were likely pushed at these sites by the southwesterly component of the wind. We also sampled inshore phytoplankton on 25 June, a day after the largest storm of the summer, with winds up to 40 $km \cdot h^{-1}$. The winds were very steady from the west (Fig. 4) and gradually decreased through the night. In the morning, surface chlorophyll concentrations ranged from 2.1 to 3.5 $\mu g \cdot L^{-1}$ and were higher at the downwind end of the basin (Fig. 4). Since the dominant winds on Lake Opeongo blow from the southwest, west, and northwest, these results suggest that more planktonic food is available to *E. complanata* living at the downwind end of South Arm, at least after windy periods.

Unionid density and body size

The density of *E. complanata* measured at 18 sampling sites in South Arm ranged from 0 to 23 individuals·m^{-2}, with a (geometric) mean density of 2.3 individuals·m^{-2}. There was no significant difference in density between the upwind and downwind sites (t test, P = 0.54), but the density of *E. complanata* was lower in areas covered with boulders (geometric mean density = 1·m^{-2}, n = 5 sites) compared with sites covered in mud or sand (4–5·m^{-2}, n = 5–6 sites; ANOVA, P = 0.08).

The unionids we collected ranged from 4.4 to 8.3 cm maximum length, and there was no difference in the length of *E. complanata* at upwind and downwind sites (Kruskal–Wallis test on growth length, P = 0.12). There was also no significant difference in *E. complanata* growth lengths among

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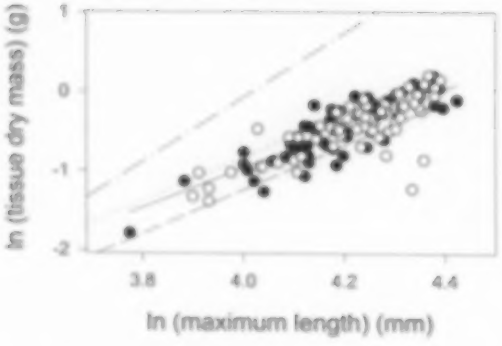
Fig. 5. Mass–length relationship for *Elliptio complanata* sampled at three upwind (open circles, $n = 74$) and three downwind (solid circles, $n = 80$) sites in Lake Opeongo. There was no significant difference in mass–length relationships between upwind and downwind sites (analysis of covariance, $P > 0.5$), so one relationship fits all unionids in South Arm: ln(soft tissue dry mass) = −11.0 + 2.5 ln(maximum length), $r^2 = 0.66$, $P < 0.0001$ (solid line). Similar relationships developed for *E. complanata* in a polymictic mesotrophic reservoir, New Brunswick, Canada (dotted line, Cameron et al. 1979), in a polymictic meso-eutrophic pond, Rhode Island, USA (dashed line, Kesler and Bailey 1993, assuming dry mass is 10% of wet mass), and in a headwater stream, Virginia, USA (dash-dotted line, Balfour and Smock 1995), are shown for comparison.



the three downwind sites (Kruskal–Wallis test, $P = 0.78$), nor among the three upwind sites ($P = 0.36$). We also used a multivariate test to compare the sizes of *E. complanata* based on maximum length, width, and height of their shells, but we did not detect any significant difference in body size between upwind and downwind sites (canonical discriminant analysis, $P > 0.2$).

The length–mass relationships of *E. complanata* were not significantly different at the upwind and downwind sites (ANCOVA, $P > 0.52$), so one relationship fits all *E. complanata*:

$$\ln(\text{soft tissue dry mass}) = -11.0 + 2.5 \ln(\text{maximum length})$$

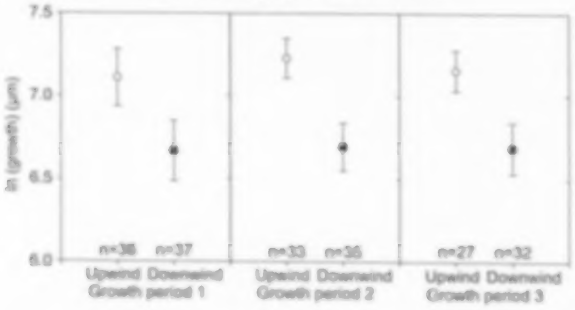
where dry mass is in g and length is in mm ($r^2 = 0.66$, $P < 0.0001$; Fig. 5).

Shell thickness and erosion

Shell thickness ranged from 0.5 to 2.8 g·cm^{-1} and did not differ significantly between the upwind and downwind ends of South Arm (ANCOVA, $P > 0.15$). These measures of shell thickness, which are calculated from shell mass and maximum shell length, should be interpreted with caution, however, since most shells were heavily eroded.

Most of the *E. complanata* shells we collected were heavily eroded, with the nacreous layer exposed over more than half of their growth length (median = 52%). Interestingly, two of the small unionids collected at site U3 (maximum length = 5.1 and 5.7 cm) showed very little erosion, with only 8%–10% of their growth length eroded. All other unionids, including other very small ones (down to 4.4 cm maximum length), had at least 20% and up to 99% of their growth length eroded. The degree of shell erosion did not increase systematically with shell length (ANCOVA, the coefficient associated with ln(growth length) ± standard error (SE) = 1.41 ± 0.24 is not significantly different from 1), but the shells were slightly more eroded (13% more erosion, on average) at downwind than at upwind sites (ANCOVA, $P = 0.02$).

Fig. 6. Growth rates of *Elliptio complanata* sampled at three upwind and three downwind sites in Lake Opeongo over the last three growth periods (growth period 1 is the most recent). *Elliptio complanata* at upwind sites consistently grew more per growth period (44% higher growth rates, on average) than at downwind sites. Symbols are mean ± standard error. Numbers of unionids measured (n) are displayed on the bottom.



Growth rate

The growth rate of *E. complanata* ranged from 0.1 to 4.5 mm per growth period and was on average 62% higher at western than at eastern sites (t tests; growth period 1, $P = 0.10$; growth period 2, $P = 0.004$; growth period 3, $P = 0.03$; Fig. 6). The growth rates of *E. complanata* were not related to their body size (regression, $P > 0.2$ in all three growth periods). When comparing growth over different periods, we found no evidence that any of the growth periods were consistently better or worse than other growth periods. We also found no evidence that individuals grew consistently more or less than others over these three growth periods. An individual with a higher-than-average growth rate in one period did not necessarily grow more than average in another period.

Food source and soft tissue composition

The δ^{13}C signatures of *E. complanata* were very similar to those of the plankton (−27.03‰ ± 0.07‰ (SE), $n = 18$ mussels from four sites; and −27.00‰ ± 0.1‰, $n = 2$ sites, respectively; Fig. 7), suggesting that their main source of food is planktonic rather than benthic. Their δ^{13}C signatures did not vary with body size (regression, $P > 0.5$), nor did we find consistent differences in δ^{13}C signatures among unionids living on sand, mud, or among boulders (circles, inverted triangles, and upright triangles, respectively; Fig. 7). The δ^{13}C signatures of *E. complanata* were closely related to their C/N ratio at downwind sites (regression, $r^2 = 0.79$, $P < 0.001$, solid symbols in Fig. 7), but not at upwind sites ($P > 0.7$, open symbols in Fig. 7). This relationship suggests that some individuals at downwind sites have low lipid content (i.e., low C/N ratio and less negative δ^{13}C; Schmidt et al. 2003; Matthews and Mazumder 2005). If we assume that

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Fig. 7. Relationship between $\delta^{13}C$ signature and C/N ratio in *Elliptio complanata* unionids at upwind (open symbols, broken line) and downwind (solid symbols, solid line) sites. Each point represents one unionid sampled on sand (open circles from site U7, solid circles from D9), mud (open triangles, site U3), or among boulders (solid triangles, site D4). The $\delta^{13}C$ signatures of plankton sampled upwind (open) and downwind (solid) are shown for comparison.

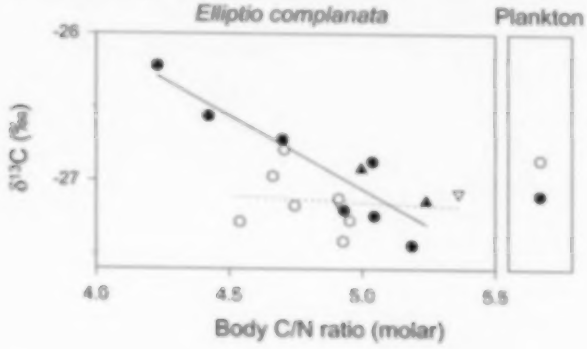
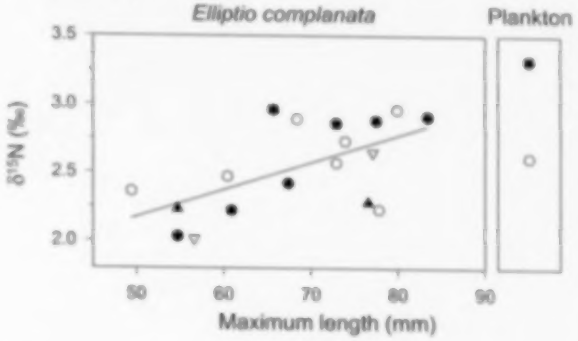


Fig. 8. Relationship between the $\delta^{15}N$ signature of soft tissue and the body size of *Elliptio complanata* collected at upwind (open symbols) and downwind (solid symbols) sites. Each point represents one unionid sampled on sand (open circles from site U7, solid circles from D9), mud (open triangles, site U3), or among boulders (solid triangles, site D4). The $\delta^{15}N$ signatures of plankton sampled upwind (open) and downwind (solid) are shown for comparison.



lipids are depleted in $\delta^{13}C$ by 3‰–12‰ (Abelson and Hoering 1961; Parker 1964; Kelly 2000) and have a high C/N ratio (Schmidt et al. 2003), the difference we observe in $\delta^{13}C$ signature and C/N ratio between unionids from downwind sites suggests a 10%–40% difference in lipid content. In contrast, the unionids at upwind sites had more similar $\delta^{13}C$ signatures that were not related to C/N ratios, suggesting more similar lipid contents. Unfortunately, we did not measure the lipid content of the unionids directly.

The $\delta^{15}N$ signatures of the *E. complanata* collected at both upwind and downwind sites in South Arm ranged over 1‰ and were similar to those measured in plankton >63 µm (Fig. 8). The $\delta^{15}N$ signatures of *E. complanata* increased with increasing body size (regression with maximum length, $r^2 = 0.34$, $P < 0.01$; Fig. 8).

Discussion

Littoral sites at the downwind end of South Arm are warmer and have higher phytoplankton biomass, at least after wind events, compared with sites at the upwind end of the basin. Higher average water temperatures, a longer growing season, and increased food availability all suggest that unionids from the downwind end of the lake should exhibit increased growth rates compared with unionids at the upwind end of the lake. Yet, we found no difference in the density or body size of unionids between the upwind and the downwind sites, and contrary to our expectations, *E. complanata* grew faster at the upwind sites, at least over the past three growth periods.

Density of *E. complanata*

The density of *E. complanata* at a depth of 2 m in South Arm ranged between 0 and 23 individuals·m^{-2}, which falls within the range of densities reported in other lakes on and off the Canadian Shield (e.g., Ghent et al. 1978; Strayer et al. 1981; Downing and Downing 1992). *Elliptio complanata* is fairly abundant in Lake Opeongo and can reach much higher densities in other basins. We have recorded densities up to 210 individuals·m^{-2} at 1 m depth in Sproule Bay, a much smaller polymictic basin at the southernmost end of Lake Opeongo (surface area = 2.1 km^2, maximum depth = 6.5 m; C.N. Hirst and H. Cyr, unpublished data).

The densities of *E. complanata* were similar at the upwind and the downwind ends of South Arm. *Elliptio complanata* move relatively short distances through the sediments (Amyot and Downing 1997), and their overall distribution may depend on the dispersal of glochidia (unionid larvae), which are obligate parasites of littoral fish (e.g., yellow perch (*Perca flavescens*), smallmouth bass (*Micropterus dolomieu*); Vaughn and Taylor 2000). In South Arm, the smallmouth bass males nest mostly at downwind sites in the spring (Rejwan et al. 1997), but yellow perch and nonnesting smallmouth bass (females, males after the nesting period) move broadly around the different basins (M. Ridgway, Aquatic Resource and Development Section, Ontario Ministry of Natural Resources, 300 Water Street, Peterborough, ON K9J 8M5, personal communication). This suggests that dispersal is not limiting the distribution of *E. complanata* in South Arm.

We found no systematic differences in the densities of *E. complanata* between muddy and sandy substrates. Areas with boulders supported fewer mussels, but this is not surprising, since the boulders reduce the effective area of quadrats that is inhabitable by mussels. Unionid densities in South Arm seem to be unaffected by substrate types, water temperature, or food availability.

Growth rate measurements

We found a clear difference in growth rates of *E. complanata* between upwind and downwind sites in South Arm. Contrary to our predictions, the growth rates were 62% higher, on average, at upwind sites.

Our comparison of *E. complanata* growth rates assumes that all unionids in the shallow littoral zone of South Arm lay their growth lines at the same time, but not necessarily at annual intervals. Internal growth lines in unionids are

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thought to be laid following extended periods of inactivity, during which the mantle retracts from the edge of the shell (Checa 2000). However, it is still unclear whether these growth lines represent annual growth or not. Veinott and Cornett (1996) showed periodic changes in $\delta^{18}O$ in the shells of five *E. complanata*, which indicates changes in water temperature as the shells were growing. Interestingly, the $\delta^{18}O$ signatures peaked in all but one of the eight growth lines tested, suggesting that the growth lines were generally produced during the coldest time of the year. Dettman et al. (1999) also found periodic changes in $\delta^{18}O$ shell signature in the shells that were associated with growth lines in three species of unionids collected in a river and a creek (one individual per species). They also showed that these changes in the shells tracked very closely the $\delta^{18}O$ signature measured in the river water over the previous 2 years. These studies suggest that unionid growth lines are mostly laid during cold periods of the year; yet this does not imply that the growth lines are laid annually. Kesler and Downing (1997) showed that when internal annuli were used to predict the growth rates of *E. complanata* and *Lampsilis radiata*, they greatly overestimated the growth rates actually measured in the field (up to 19 times higher). Kesler and Downing (1997) concluded that internal annuli cannot be produced every year (see also Anthony et al. 2001).

The growth rate differences we measured between upwind and downwind sites were relatively large and consistent among the last three growth periods. Nevertheless, we did not detect differences in shell length or general body size (using multivariate analysis) of *E. complanata* between upwind and downwind sites. The most likely reason for this inconsistency is that the absolute difference in growth of *E. complanata* between upwind and downwind sites is small (0.5 mm per growth period, on average), so it would take years to detect differences in shell length, especially if growth lines are not produced annually. Another possible explanation is that unionids of a given size are not all of the same age. The faster-growing unionids at upwind sites may be a younger cohort than the slower-growing unionids downwind. There is currently no reliable method to age these heavily eroded unionids, so we cannot test this hypothesis. Finally, we do not know whether the growth rates we measured on unionids >4 cm in length are representative of earlier growth. If young *E. complanata* remain buried for long periods of time (Amyot and Downing 1991), their size may not differ much between upwind and downwind sites.

Why are *Elliptio* growth rates higher at upwind sites?

Large differences in water temperature were observed between the upwind and downwind littoral sites in South Arm. Downwind sites were often 2–5 °C warmer than upwind sites, especially during spring and early summer, with differences as large as 10–13 °C in 1998. These temperature differences are due to upwellings of cold hypolimnetic waters at upwind sites during windy events (Finlay et al. 2001). These upwelling events can last several hours and sometimes days. The emergence of *E. complanata* from the sediments in the spring is strongly correlated with increasing water temperature (Amyot and Downing 1997; Watters et al. 2001), so we expected these frequent cold water incursions at upwind sites to shorten the growing season of unionids and (or) to reduce their growth rate. Nevertheless, we found the opposite; the growth of unionids was higher at upwind sites.

Temperature refuge in the sediments?

One possible explanation for this apparently puzzling result is that temperature of the water column may not represent the temperature experienced by unionids, especially early in the season when they are still buried in the sediments. In July 2003, we deployed two Stowaway temperature loggers (model TBI32-05+37; Hoskin Scientific) at a 5 m deep site for 1 month to measure temperature at 8 min intervals simultaneously in the water column (20 cm above the sediments) and 1 cm into the sediment. Our data suggest that upwellings of cold water were also measured in the sediments. Temperature variability in the sediments was more dampened at short time scales (<2 h) compared with temperature in the water column, but was equally variable at longer time scales (H. Cyr, unpublished data). It is unclear whether these results can be extrapolated to earlier periods of the year, to shallower littoral sites with different substrate composition, or to deeper sediments where the unionids are buried, but they suggest that prolonged cold-water upwellings penetrate into the sediments. The effect of upwellings of cold water on unionids buried in the sediment remains to be tested.

Food availability?

The stable isotope analysis establishes that *E. complanata* feed on a similar C source as the planktonic organisms larger than 63 µm. The $\delta^{13}C$ signatures of *E. complanata* and of planktonic organisms are both around –27‰. These results are similar to the signature of pelagic primary consumers in other Canadian Shield lakes (–28‰, on average) and are clearly different from the signature of benthic primary consumers (–24‰, on average; Vander Zanden and Rasmussen 1999). *Elliptio complanata* also has a $\delta^{15}N$ signature that is similar to that of planktonic organisms collected with a 63 µm mesh (mostly crustacean zooplankton, with some large colonial algae), suggesting that they are feeding on similar food sources. Paterson (1986) reported that *E. complanata* fed best on very small seston (between 1.6 and 5 µm in diameter), and their efficiency decreased with increasing particle size. These feeding experiments, however, were run in the laboratory under crowded conditions (five *E. complanata* in 6 L of water). In contrast, Bärlocher and Brendelberger (2004) reported particle-specific rather than size-specific feeding of *E. complanata*. In their experiment, *E. complanata* preferred three large species of conidia (asexual spores of fungi), ranging in size between 70 and 200 µm. Contrary to zebra (*Dreissena polymorpha*) and quagga (*Dreissena bugensis*) mussels, *E. complanata* has not been observed to feed on rotifers (Thorp and Casper 2002).

The increased availability of phytoplankton nearshore at downwind sites should be available to the unionids, since the water column in littoral areas should be well mixed, especially after wind events. Our results suggest that despite low phytoplankton biomass in South Arm (<5 µg chlorophyll a·L^{-1}), *E. complanata* does not appear limited by food availability, or at least any subsidy in food downwind is confounded by other factors that affect their growth negatively. Alternatively, unionids at downwind sites may

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not be able to use these intermittent food pulses, for example, if increased sediment resuspension after storms reduces food quality for the filtering unionids (Grant and Thorpe 1991; Ellis et al. 2002).

Exposure to wave action?

South Arm is 5.8 km in length and its main axis is aligned with the prevailing winds. The unionids we sampled at the downwind sites are exposed to large wave action during storms and may be adversely affected by these conditions. In very large lakes, the size of unionids can be stunted by exposure to extreme wave action. Brown et al. (1938) found that *Lampsilis radiata* were stunted in highly exposed habitats in western Lake Erie, Ontario. Green (1972) reported decreasing sizes of *L. radiata* with increasing lake size in large Canadian lakes (maximum fetch: <2 to 140 km). In contrast, exposure to wave action in smaller and more protected basins has been reported to enhance growth. Hinch and Bailey (1988) compared the morphometrics of *E. complanata* in four relatively small lakes (surface area = 1.2–1.7 km^2) and found that they were consistently larger and heavier in areas of highest exposure within these lakes. Heavier shells were hypothesized to prevent dislodgement under turbulent conditions. Hinch et al. (1986) also found, through reciprocal transplant experiments between two sites in Inner Long Point Bay, Lake Erie (surface area of 75 km^2), that *L. radiata siliquoidea* had a different shape and grew faster at the more exposed site. These differences were attributable partly to environmental differences between the sites and partly to genetic differences between the two populations. The South Arm of Lake Opeongo is intermediate in size (22 km^2), and although we did not detect any differences in the size or shape of *E. complanata* between upwind and downwind sites, we measured clear differences in growth rates, with higher growth rates measured at the least-exposed upwind sites.

There are two lines of evidence suggesting that *E. complanata* growing at downwind sites may be more stressed. First, although most *E. complanata* in South Arm have heavily eroded shells, those we collected downwind were slightly more eroded than those from upwind sites (13% more eroded, on average).

Second, the $\delta^{13}C$ signature and C/N ratio of mussel soft tissues suggest that some mussels from shallow downwind sites have low body lipid content. The mussels collected at downwind sites showed a clear negative relationship between their $\delta^{13}C$ signature and their C/N ratio. Other studies report similar relationships for other groups of organisms (e.g., Matthews and Mazumder 2005 for zooplankton; Cherel et al. 2005 for penguins), which they attribute to differences in lipid content. Lipids have a less negative $\delta^{13}C$ signature (and a lower C/N ratio) than do carbohydrates or proteins (deNiro and Epstein 1977), and depending on the fractionation difference one assumes for lipids (3‰–12‰; Abelson and Hoering 1961; Parker 1964; Kelly 2000), the 1.2‰ difference in $\delta^{13}C$ signature we observed among unionids at downwind sites suggests a 10%–40% difference in lipid content. Lipid content is also positively related to the C/N ratio of the organisms (e.g., Schmidt et al. 2003), and the 1.1 difference in C/N ratio we observed at downwind sites also suggests a 10% difference in lipid content. Low body lipid content is an indicator of poor condition in organisms (e.g., Hebert et al. 1991; Greseth et al. 2003) and could be due to low feeding rates (e.g., from physical disturbance of the mussels) or low food quality (e.g., because of sediment resuspension during wind events). This conclusion needs to be tested more directly by comparing the physiological condition of these unionids.

***E. complanata* $\delta^{15}N$ increases with body size**

Most studies show that $\delta^{15}N$ is not related to the size of organisms (Kelly 2000). This includes an early study of stable isotopes in marine mussels, where $\delta^{15}N$ was compared specifically between individuals of different sizes (Minagawa and Wada 1984). In this study, it was assumed that the planktonic food filtered by these mussels did not change during the sessile part of their lives. This might not be strictly the case, since phytoplankton and zooplankton are transported differently by water currents, and their availability onshore may vary with changes in wind conditions and water currents. Interestingly, in Lake Opeongo we found no systematic difference in $\delta^{15}N$ (for a given body size) between upwind and downwind sites, suggesting that wind-induced shifts in the distribution and availability of planktonic organisms do not affect the $\delta^{15}N$ signature of the unionids.

A few studies have reported increases in $\delta^{15}N$ with increasing body size, which they attribute to ontogenic shifts of diet (e.g., in crabs, Wada et al. 1993; in fish, Post 2003; in squids, Cherel and Hobson 2005). *Elliptio complanata* is a filter feeder, and although we actually know very little about its feeding, it is not known to shift its diet with increasing body size. Instead, Post (2002) and Garton et al. (2005) attributed the high $\delta^{15}N$ measured in large (and presumably old) bivalves to a carryover effect from previous years. It is unlikely that the higher $\delta^{15}N$ signature of large unionids in our study could be attributed to recent changes in the lake. Lake Opeongo is located in Algonquin Provincial Park (established in 1893). The lake and its watershed have been protected from major human development, and although the introduction of smallmouth bass (early 1900s) and cisco (*Coregonus artedi*) (1940s) and recreational fishing had important effects on the lake food web (Martin and Fry 1972), there has been no major changes recorded in the lake over the last 30–40 years (St. Jacques et al. 2005). The increase in $\delta^{15}N$ with increasing body size suggests that *E. complanata* may shift its diet as it grows, from a more phytoplankton-based diet to a more zooplankton-based diet. However, many other factors could also affect the $\delta^{15}N$ signature of organisms (Kelly 2000; Vanderklift and Ponsard 2003). For example, $\delta^{15}N$ has been shown to increase in fasting and starving organisms (Adams and Sterner 2000; Cherel et al. 2005), and low food availability in oligotrophic lakes may progressively affect the $\delta^{15}N$ of *Elliptio complanata* as they grow older. At this point, it is unclear why we are finding increases in $\delta^{15}N$ with increasing body size, but it clearly has implications for how we use stable isotopes in food web studies.

Unionids are commonly used to establish a $\delta^{15}N$ baseline in freshwater food webs (Cabana and Rasmussen 1996; Vander Zanden and Rasmussen 1999; Post et al. 2000). Their large size results in a slow nitrogen turnover rate and low temporal variation in their $\delta^{15}N$ signatures compared with phyto-

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plankton or zooplankton (Cabana and Rasmussen 1996). Studies currently using unionids to establish their trophic baseline generally rely on one or very few individuals to establish the baseline, implicitly assuming negligible variation in unionid $\delta^{15}N$ signatures within a lake. This assumption was tested in six small lakes by McKinney et al. (1999; mean depth = 1.2–3.6 m, surface area = 0.2–1 km^2), who reported that the $\delta^{15}N$ signature of unionids sampled at different sites within a lake or pond varied by 0.7‰–1.9‰. This represents a maximum error of 0.5 to almost 1 trophic level, depending on the fractionation between trophic levels (2.5‰–3.4‰; Minagawa and Wada 1984; Post 2002; Vanderklift and Ponsard 2003). In South Arm, we found a maximum difference of 1‰ in $\delta^{15}N$ signatures of *E. complanata*. Our results support the finding of McKinney et al. (1999) in a much larger lake basin (mean depth = 14.6 m, surface area = 22.1 km^2), with obvious habitat heterogeneity.

This variability in $\delta^{15}N$ signature is related to the body size (soft tissue dry mass) of *E. complanata*. Larger mussels have up to a 1‰ higher $\delta^{15}N$ signature. Although the differences we found are small, it would be prudent to standardize the size of the unionids used as baseline (e.g., 6–7 cm length) when constructing food webs using $\delta^{15}N$ signatures.

Elliptio complanata showed similar densities and body sizes at upwind and downwind sites in South Arm, but individuals at the upwind sites grew 62% faster. These results are opposite to our initial predictions, which were based on differences in temperature and food conditions at both ends of the basin. They raise numerous questions on how *E. complanata* responds to water temperature and to food availability. High growth rates do not translate into differences in body size in this unionid population. This suggests that the *Elliptio* "hot spots" we identified represent a population of very slow-growing unionids living in a low productivity lake.

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